

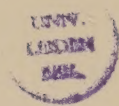
NUTRITIONAL PROPERTIES OF WHEAT PRODUCTS
PROCESSED BY
HTST EXTRUSION COOKING

Ingemar Björck



Lund 1984

STATIONARY ENGINEER'S REPORT
ON THE
WORKING



**NUTRITIONAL PROPERTIES
OF WHEAT PRODUCTS
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HTST EXTRUSION COOKING**

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PAPERS

This thesis is based on the following papers which will be referred to in the text by their Roman numerals.

- I Protein Nutritional Value of a Biscuit Processed by Extrusion Cooking: Effects on Available Lysine
Björck, I., Noguchi, A., Asp, N.-G., Cheftel, J.-C. & Dahlqvist, A.
J. Agric. Food Chem. **31** (1983) 488 - 492
- II Protein Nutritional Value of Extrusion-Cooked Wheat Flours
Björck, I., Asp, N.-G. & Dahlqvist, A.
Food Chem., accepted for publication
- III Effects of Processing on Starch Availability IN-VITRO and IN-VIVO: Extrusion Cooking of Wheat Flours and Starch
Björck, I., Asp, N.-G., Birkhed, D. & Lundquist, I.
J. Cereal Sci., in press
- IV Effects of Processing on Starch Availability IN-VITRO and IN-VIVO: Drum-Drying of Wheat Flour
Björck, I., Asp, N.-G., Birkhed, D., Eliasson, A.-C., Sjöberg, L.-B. & Lundquist, I.
J. Cereal Sci., in press
- V Hormonal and Metabolic Responses to Breakfast Meals in NIDDM: Comparison of White and Whole-grain Wheat Bread and Corresponding Extruded Products
Hagander, B., Björck, I., Asp, N.-G., Lundquist, I., Nilsson-Ehle, P., Schrezenmeir, J. & Scherstén, B.
submitted for publication
- VI Extrusion Cooking and Dietary Fiber: Effects on Dietary Fiber Content and on Degradation in the Rat Intestinal Tract
Björck, I., Nyman, M. & Asp, N.-G.
Cereal Chem., in press

	Page
1 INTRODUCTION	6
2 HTST EXTRUSION COOKING	
2.1 Basic Principles and Applications	7
Literature Cited 2.1	12
2.2 Effects of Extrusion Cooking on Nutritional Value -	
A Literature Review	14
3 PURPOSE AND DESIGN OF THE PRESENT STUDY	42
3.1 Extrusion Equipment and Process Conditions	44
4 RESULTS AND DISCUSSION	
4.1 Protein Nutritional Value	46
4.1.1 Lysine retention	47
4.2 Starch Availability	50
4.2.1 Digestion with salivary amylase <u>in vitro</u>	51
4.2.2 Fermentation in human dental plaque <u>in situ</u>	53
4.2.3 Plasma glucose and insulin responses in rats	54
4.2.4 Post-prandial glucose and hormonal responses in	
non-insulin-dependent diabetics	55
4.3 Dietary Fibre	56
4.3.1 Dietary fibre content	57
4.3.2 Fermentation in the rat intestine	58
Literature Cited 4.1 - 4.3	60
5 SUMMARY	68
6 ACKNOWLEDGEMENTS	69
7 PAPERS	
I	71
II	77
III	97
IV	111
V	141
VI	163

HTST (high-temperature, short-time) extrusion cooking can be described as a continuous process for cooking and forming mainly cereal based products. The extruder is somewhat unique compared with other systems for heat treatment of food, in that the cooking takes place at low water content. Further, the material is subjected to intense mechanical shear, resulting in a complete disorganization of the structure present in the raw material.

During the last decade there has been a great increase in the number of applications for extrusion cookers. At present many different types of products are produced with this technique. Due to the high productivity and the low floor-space requirement, extruders provide an economic alternative for the pre-cooking of starches and cereal flours. Extrusion cooking is also used as a continuous baking process.

In certain types of extruded products, e.g. weaning food, gruel and bread, a high retention of nutrients is essential. Several reports are available on nutrient retention during HTST extrusion cooking. However, many studies have been performed without satisfactory characterization of the process conditions used. Furthermore, most reports concern single-screw extruders. Twin-screw extruders are now increasingly being used due to their greater versatility. In addition, this type of extruder allows extremely dry materials to be processed.

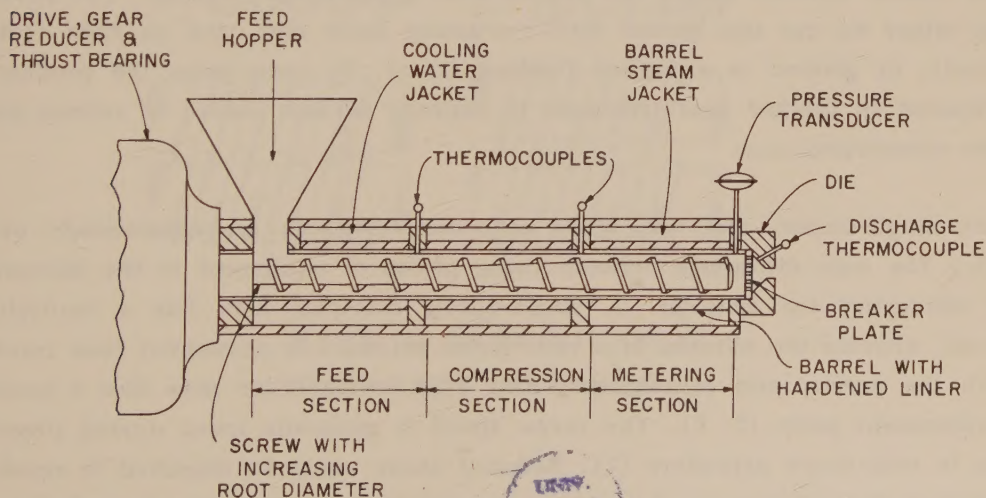
The present work reports data on the effect of HTST twin-screw extrusion cooking on some nutritional properties of wheat products.

2.1 BASIC PRINCIPLES AND APPLICATIONS

A cooker extruder allows low-moisture ingredients to be cooked continuously at high temperatures and pressures, without the addition of water. The process temperature can be up to 250°C . The residence time at high temperature is very short, 5 to 10 s, and extrusion cooking can therefore be classified as a HTST-treatment (1, 2, 3).

A food extruder can be described as a pump that simultaneously transports and mixes flours or granular materials (Fig. 1). The extruder device consists of a flighted screw, which rotates in a tightly fitting barrel. The screw can either be in one single piece or consist of separate interchangeable pieces (4). The screw flights push the material towards a die at the discharge end of the barrel. The heat generated through viscous dissipation of mechanical energy may be sufficient to cook the product, or external heat can be supplied through heating elements surrounding the barrel. In some cases, cooling is necessary to obtain proper temperature control. The combined effect of temperature, pressure and shear, transforms the product into a viscous dough-like mass, which is then pressed through the die.

Fig. 1 Single-screw extruder



from Harper, 1981 (1)

The extruder is often divided into three sections, which are not sharply defined, but useful in order to visualize the process. The first section, where the raw material is introduced, can be described as a solid-conveying zone. In this zone the screw is only partially filled, and no pressure is developed. Eventually, the screw is filled with material, and there is a rise in pressure and temperature. In this section, the compression section, the viscosity changes due to the onset of protein denaturation and gelatinization of starch. Subsequently, in the cooking or metering section, the material is transformed into a homogeneous melt, and the viscosity and heat dissipation increase rapidly. The fundamental physical and chemical transformations occur in this zone. In spite of the low moisture content, complete gelatinization of starch and denaturation of protein are possible.

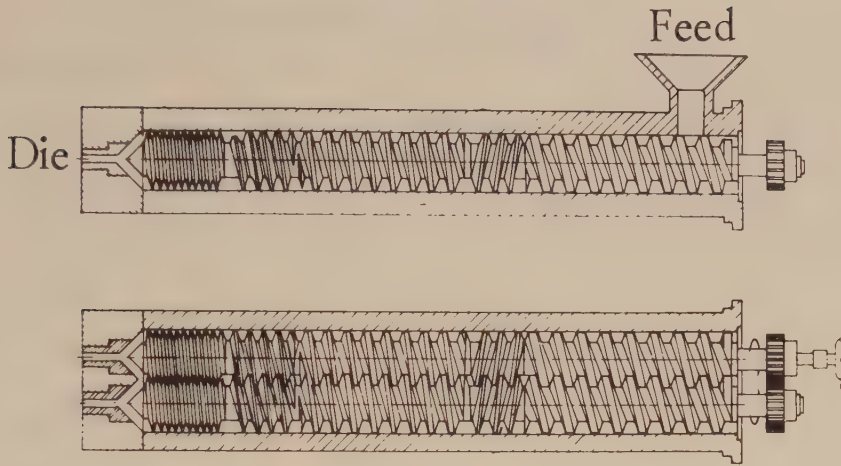
The pressure and temperature profiles in the extruder are dependent on several parameters e.g. screw- and die geometry, flow restrictions (reverse pitch screw elements, breaker bolts) and external heating or cooling.

When the product emerges through the die, superheated vapour flashes off due to the instant drop in pressure. The dough-like mass expands and settles in a highly porous structure. The die is often designed to obtain a special product shape. The loss of moisture when passing the die, results in adiabatic cooling (1, 5). Thus, in an extruder, cooking, forming, drying and cooling can be achieved in one single processing step.

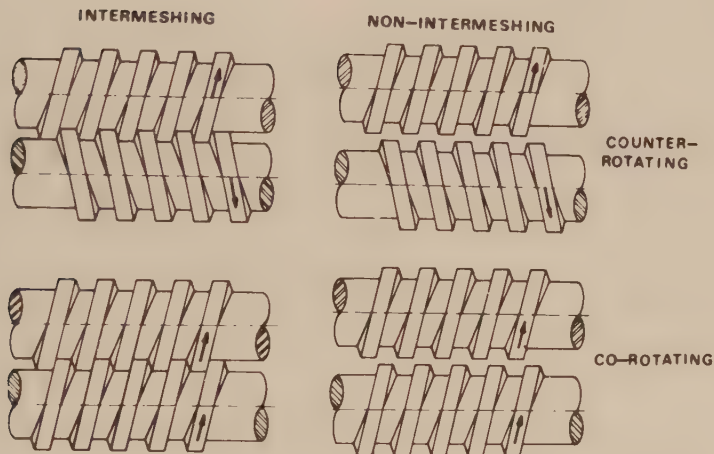
The cooked material leaves the extruder as a continuous ribbon. The product can either be cut into pieces with a rotating knife and used as such (crisp-bread), or ground to a powder (instant flour). In some cases the product is subjected to further heat treatment to improve surface colour or reduce moisture content.

Both single-screw- and twin-screw extruders (Fig. 2) are commercially available. The main difference between these pieces of equipment is the mechanism of conveying the material. A single-screw extruder acts like a centrifugal pump, whereas the material in a twin-screw extruder is prevented from rotating with the screw, and consequently this type of extruder acts like a positive displacement pump (3, 6). The screw speed is generally lower during processing in twin-screw extruders (7). Reduced shear would be expected to result in a better thermal homogeneity of the material being processed. However, additional shear is often introduced in twin-screw extruders by inserting reverse-flight screw elements (8), and it has been suggested that the conventional

Fig 2. Twin-screw extruder



Side and top views



Screw configurations in a twin-screw extruder

(from El-Dash, 1981 (4))

classification of twin-screw extruders as low shear systems, should be reconsidered (9).

Single-screw cooker extruders have been used since the late 1940's, whereas the twin-screw extruder was introduced more recently (1). The twin-screw extruder has a greater versatility, and is capable of working over a wider range of moisture content. Extremely dry materials can be processed, which makes after-drying unnecessary (3).

Typical extrusion conditions during processing in an HTST twin-screw extruder are given in the table below (Table 1).

Table 1

Process conditions during HTST twin-screw extrusion cooking

Process temperature	80 - 250 ⁰	C
Pressure	2 - 20	MPa
Moisture content	> 5	%
Screw speed	10 - 250	rpm
Residence time	5 - 120	s

By changing the recipe of the ingredient mixture and/or the process conditions, a great variety of products can be obtained (1, 3). Extrusion cookers are used at present for e.g. continuous production of bread products, pre-cooking of cereal flours, forming of breakfast cereals and inactivation of trypsin inhibitors in soy bean flour. The ability of long molecules to align themselves in the shear field in the extruder, is utilized for texturization of plant proteins for use as meat substitutes.

The productivity of cooker extruders is high compared to that with other processes for cooking of food. As low moisture materials can be processed, less heat is required for cooking and redrying after cooking. According to El-Dash (4) and Ben-Gera et al. (10), extrusion processing is both technologically and economically advantageous over drum-drying for pre-cooking of starches and flours.

Present and potential applications of extrusion cooking in the food industry are listed in Table 2 (11).

Table 2

Present and potential applications of extrusion cooking

CEREAL PRODUCTS

- * doughs for pasta, bread & tortillas
- * crispbread, bread crumb
- * biscuits, crackers, wafers & croutons
- * breakfast cereals
- * pre-gelatinized starches
- * pre-cooked flours and grits
- * pre-treated malt for brewing
- * instant rice pudding

POTATO PRODUCTS

- * analogues of pommes frites
- * purée

DAIRY PRODUCTS

- * sterile cheese

VEGETABLES & PULSES

- * textured proteins (meat analogues)
- * full-fat soy flour

MIXED PRODUCTS

- * different types of co-extruded ravioli
- * instant dry soup mixes

NUTRIENT ENRICHED PRODUCTS, WEANING FOOD & BABY FOOD

- * Corn-Soy-Milk
- * Whey-Soy-Blend
- * pre-cooked instant gruels
- * fruit-, vegetable- & meat purées

SNACKS

LITERATURE CITED (2.1)

1. Harper, J.M. (1981) Extrusion of Foods. Vol 1. CRC Press Inc., Boca Raton, Florida.
2. Olkku, J., Antila, J. and Heikkinen, J. (1980) Residence time distribution in a twin-screw extruder. In: Food Process Engineering. Vol 1. Food Processing Systems (P. Linko, Y. Mälkki, J. Olkku and J. Larinkari, eds.) Applied Science Publishers Ltd, London, pp 791 - 794.
3. Linko, P., Colonna, P. and Mercier, C. (1981) High-temperature, short-time extrusion cooking. In: Advances in Cereal Science and Technology, Vol IV. (Y. Pomeranz, ed.), American Association of Cereal Chemists Inc., St. Paul, Minnesota, pp 145 - 235.
4. El-Dash, A.A. (1981) Application and control of thermoplastic extrusion of cereals for food and industrial uses. In: Cereals - A Renewable Resource, Theory and Practice. (Y. Pomeranz and L. Munck, eds.), American Association of Cereal Chemists Inc., St. Paul, Minnesota, pp 165 - 216.
5. Fabion, J.M., Hosney, R.C. and Seib, P.A. (1982) Cereal Foods World 27, 212 - 216.
6. van Zuilichem, D.J., Albas, B., Reinders, P.M. and Stolp, W. (1983) A comparative study of the operational characteristics of single and twin screw extruders. Abstract. COST 91. Concluding Seminar "Thermal Processing and Quality of Foods", Athens 14 - 18 November, Greece.
7. van Zuilichem, D.J. and Stolp, W. (1982) New twin screw extruder equipment for food extrusion. Paper presented at the International Seminar "Cooking and Extruding Techniques", ZDS, Central College of the German Confectionary Trade/Solingen-Gräfrath, Solingen 11 - 13 October, W. Germany.
8. Jansen, L.P.B.M. (1982) Screw lay-out for twin screw extruders. Paper presented at the International Seminar "Cooking and Extruding Techniques", ZDS, Central College of the German Confectionary Trade/Solingen-Gräfrath, Solingen 11 - 13 October, W. Germany.
9. Colonna, P., Melcion, J.-P., Vergnes, B. and Mercier, C. (1983) J. Cereal Sci. 1, 115 - 125.

10. Ben-Gera, I., Smith, O.B. and Rokey, G.J. (1983) Energy aspects in extrusion cooking of starches and flours. Abstract. COST 91. Concluding Seminar "Thermal Processing and Quality of Foods", Athens 14 - 18 November, Greece.
11. Cheftel, J.-C. (1983) Industrial applications of extrusion cooking - present or potential. Abstract. COST 91. Concluding Seminar "Thermal Processing and Quality of Foods", Athens 14 - 18 November, Greece.

2.2 The Effects of Extrusion Cooking on Nutritional Value – A Literature Review*

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ABSTRACT

Like other processes for heat treatment of food, extrusion cooking may have both beneficial and undesirable effects on nutritional value. Beneficial effects include destruction of antinutritional factors and gelatinization of starch. On the other hand Maillard reactions between protein and sugars reduce the nutritional value of the protein. Heat-labile vitamins may be lost to varying extents.

In this paper a review of the literature is presented. The type of extruder is specified and, when relevant and known, the process conditions.

INTRODUCTION

Extensive reviews on extrusion cooking have been published recently by Harper (1979, 1981) and by Linko *et al.* (1981). Time-temperature conditions are comparable to other high-temperature, short-time (HTST) processes. HTST processing is considered preferable in terms of nutrient retention, since growth inhibitors and contaminating microorganisms are more effectively destroyed. However, in extrusion cooking some unique features are present because the product is subjected to high pressure in combination with severe shear. The effect of these conditions on nutrients is not well documented. The degree of mixing and homogenization during extrusion cooking might decrease diffusion barriers or break chemical bonds, and thus increase reactivity in a food system. Since extrusion cooking is used increasingly for

* Presented at the EXTRUSION COOKING Symposium, 7th World Cereal and Bread Congress, Prague, June/July 1982.

production of weaning foods, breakfast cereals and bread-type products, a thorough knowledge of the effects on nutritional value is essential. When evaluating extrusion cooking, comparisons should be made with alternative processes such as drum-drying, boiling, autoclaving and baking.

A number of difficulties arise when trying to summarize published data. Firstly, different kinds of extruders are used. Secondly, processing conditions are not always well-defined. Thirdly, the processing conditions are interrelated, making it difficult to relate nutrient retention to any one single factor. Caution must also be used when transferring results from one food system to another.

The process conditions considered are as follows: extruder type; mass temperature, t_m , or barrel temperature, t_b ($^{\circ}\text{C}$); moisture content, w/w ($\text{H}_2\text{O}\%$); feed rate (g min^{-1}); screw speed (rpm); screw compression ratio, c.r.; die diameter, ϕ (cm); residence time, T_r (min or s); pressure (MPa); torque (Nm).

PROTEIN

Mild heat treatment of vegetable proteins generally improves digestibility due to inactivation of protease inhibitors and other antiphysiological substances. With increasing severity of heat treatment, protein digestibility decreases. The biological availability of the amino acids may be affected through different mechanisms: the sulphur-containing amino acids are sensitive to oxidation and desulphurization; heating in the presence of reducing sugars leads to decreased availability of lysine, in particular through the Maillard reaction (Hurrell and Carpenter, 1977).

The nutritional value of protein after processing in single-screw extruders changes little. Examples of such products are full-fat soy flour and precooked cereal-based blends for use in child or maternal feeding programmes (Jansen *et al.*, 1978; Lukoo, 1980; del Valle *et al.*, 1981; Harper and Jansen, 1981). Fewer biological data are available on the effects of twin-screw extruders which are able to operate over a wider range of moisture content (Linko *et al.*, 1981).

Inactivation of protease inhibitors

Its high protein content and well-balanced amino acid pattern make soy bean a valuable protein source in the human diet. However, growth

inhibitors present in raw beans must be inactivated. For example, protease inhibitors have a harmful effect on the digestion of soy protein by blocking proteolytic enzymes (Krogdahl and Holm, 1979) and conventionally, they are inactivated by direct-steam heat treatment (Horan, 1966).

In recent years, several authors have described the use of cooking extruders in processing full-fat soy flour (FFSF). Mustakas *et al.* (1970) determined optimum extrusion conditions for processing FFSF. Before processing, the soy beans were preconditioned using dry heat. The destruction of trypsin inhibitors (TI) increased with extrusion temperature and moisture content. At constant temperature, inactivation increased with the product of residence time and moisture content. The highest protein quality, as measured by PER (corrected for a value for casein of 2.5), was 2.15, obtained at a barrel temperature of 153°C, 20% moisture and 2 min residence time. Under these conditions the inactivation of TI was 89%. A pilot-plant plastics extruder was used with $t_b = 139\text{--}167^\circ\text{C}$, $\text{H}_2\text{O} = 15\text{--}30\%$ and $T_r = 0.5\text{--}2$ min. In another study, without preconditioning prior to extrusion cooking (Lorenz *et al.*, 1980), a temperature of 143°C produced a product of maximum PER despite the finding that only 57% of TI was destroyed. Urease, often used as an index of adequate heat treatment, was more readily destroyed (Brady low-cost extruder, $t_b = 121\text{--}149^\circ\text{C}$). An increase in feed rate with otherwise similar process conditions has been reported to result in less destruction of trypsin inhibitors (Köhler, 1981) presumably because of reduced residence time.

Availability of lysine in extrusion-cooked FFSF was only slightly reduced, while TI and urease activity were effectively destroyed in experiments by Bookwalter *et al.* (1971a) ($t_b = 139\text{--}167^\circ\text{C}$, $\text{H}_2\text{O} = 15\text{--}20\%$, $T_r = 1.25\text{--}2$ min). Mustakas *et al.* (1964) found that extruded FFSF compared very favourably with the conventionally-processed product. In this study direct steam was used to adjust feed moisture content. The biological value measured in chicks indicated a higher availability of sulphur-containing amino acids in extrusion-cooked FFSF. Available lysine was also higher than in conventionally-processed FFSF. Barrel temperatures in the range 133–139°C were sufficient to inactivate 95% or more of the TI present (Wenger single-screw, $t_b = 133\text{--}161^\circ\text{C}$, $\text{H}_2\text{O} = 18\text{--}20\%$, $T_r = 1\text{--}1.5$ min).

In summary, extrusion cooking appears to be a suitable process for production of full-fat soy flour products of optimal protein quality.

Extruded full-fat soy flour products have been evaluated as supplements to cereals in weaning foods (Jansen *et al.*, 1978; Jansen and Harper, 1980), for fortification of bread and biscuits (Bookwalter *et al.*, 1971b; Tsen *et al.*, 1975) and for production of soy beverage powder (Mustakas *et al.*, 1971). Available data indicate that these products have good nutritional and functional properties. Griensen (1981) summarized a number of applications for extrusion-cooked FFSF in order to produce nutritious foods at low cost.

The Maillard reaction (non-enzymic browning)

The process conditions used in extrusion cooking – high temperatures in combination with low water content – are known to favour the Maillard reaction (Lea and Hannan, 1949). This reaction between reducing sugars and free amino groups in proteins leads to a decrease both in protein digestibility and in availability of amino acids involved.

Dry heat treatment as in roasting or baking is more damaging than autoclaving or pressure cooking where water activities are close to 1 (Adrian, 1974). Maximum browning occurs at water activities between 0.3 and 0.7 depending on the type of food (Eichner, 1975). Pentoses are most reactive, followed by hexoses and disaccharides (Rubenthaler *et al.*, 1963). Lysine is the most reactive protein-bound amino acid due to its free ϵ -amino group. However, arginine, tryptophan, cysteine and histidine may also be affected (Hurrell and Carpenter, 1977). Available methionine may decrease considerably during autoclaving of casein in the presence of reducing sugars (Rao and McLaughlan, 1967). Since lysine is limiting in cereal proteins, the fate of this amino acid during extrusion cooking is especially important. In addition to a decrease in nutritional value, some data indicate that the Maillard reaction may produce toxic compounds (Adrian, 1974).

Lysine losses under extrusion-like conditions

Reduction in the availability of lysine during processing can be divided into three phases (Jokinen *et al.*, 1975). The first one follows first order kinetics and is rapid. In the second phase, the transition zone, there is some increase in available lysine with increasing processing time. After this short recovery the available lysine level stabilizes in a third phase (no-loss phase) with no further losses of lysine.

Thompson *et al.* (1976) studied lysine retention in a soy protein system under extrusion-like conditions, that is at $a_w = 0.65$ and temperatures between 80 and 130°C. The samples were heated in sealed pouches for 0.5–680 min (isothermal unstirred conditions). The increase in available lysine (FDNB reactive) during the transition zone was statistically significant, and has also been verified in animal experiments (Wolf *et al.*, 1979). Under unstirred isothermal conditions the third phase occurs at 35–55% loss (Wolf *et al.*, 1978).

Efforts have been made to develop mathematical models for the prediction of lysine losses during heat treatment of foods. In model systems Jokinen *et al.* (1975) and Thompson *et al.* (1976) found that sucrose and starch levels were not significant predictors of lysine loss, when water was measured as moisture content. These authors suggest that the effect of these non-reducing carbohydrates on lysine retention is due to their water binding activity. However, during true extrusion cooking, reducing carbohydrates are formed by hydrolysis of starch and sucrose, thus increasing reactivity (Mercier, 1977; Sahagun and Harper, 1980; Noguchi *et al.*, 1982). The third phase occurs in model systems under both unstirred and stirred conditions. In contrast, during extrusion cooking the third phase does not always occur, and the loss of available lysine may continue beyond that predicted from model systems (Wolf *et al.*, 1978). When a model system has entered the third phase, application of pressure or shear does not cause the system to revert to the rapid-loss phase (Wolf *et al.*, 1977).

The importance of these differences between model systems and extrusion cooking from a nutritional point of view is difficult to establish. When processing foods, a 35–55% loss (third phase) is already a considerable loss. According to Harper (1979), only the first-order reaction needs to be considered during extrusion cooking since the residence time is normally very short. The mechanism behind the transition zone and the third phase is not known and is an interesting field for further research.

Losses of available lysine during extrusion cooking

Beaufrand *et al.* (1978) studied the influence of different carbohydrates. In a cereal mixture the loss of available lysine ranged from 32 to 80% (Creusot-Loire BC 45, $t_m = 170^\circ\text{C}$, $\text{H}_2\text{O} = 10$ or 14%, 60 rpm). The order of reactivity was fructose > honey > sucrose > sorbitol. Even in

the cereal mixture extruded without addition of sugars, the loss of available lysine was substantial – 32% – possibly due to hydrolysis of starch. Similarly sucrose increased lysine degradation whereas sorbitol did not, possibly due to the breakdown of sucrose to reducing sugars. Modification of screw geometry did not improve lysine retention. However, an increase in moisture content (from 10 to 14%) significantly reduced the lysine loss from 40 to 10% in the cereal/sucrose mixture.

Köhler (1981) compared the reactivity of hexoses, pentoses and disaccharides. Sucrose, maltose and fructose were much less reactive than glucose under similar extrusion conditions. At a low hexose content (1–5%) there was selective damage to lysine. Hexoses were less reactive than pentoses. At a high energy input to the extruder, glucose caused losses of available lysine and arginine of 61 and 15%, respectively. In contrast, with xylose the losses were greater – 70 and 32%, respectively – and there was a general decrease in amino acid availability as well as in protein digestibility.

Noguchi *et al.* (1982) studied the retention of available lysine (FDNB reactive) during processing of a cereal/soy-based mixture containing 20% sucrose (Creusot-Loire BC 45, $t_m = 170\text{--}210^\circ\text{C}$, $\text{H}_2\text{O} = 13\text{--}18\%$, 40–80 rpm, $T_r = 60\text{--}71\text{ s}$). Depending on extrusion conditions lysine loss ranged from 0 to 40%. The loss increased with temperature and decreasing moisture content of feed. No effect of screw speed on lysine was seen. The extruder was operated with a reversed-flight screw element located near the die and the median residence time was not affected by screw speed over the range studied. Formation of reducing monosaccharides through sucrose hydrolysis was suggested as the rate-limiting step in lysine degradation. The highest yield of reducing sugar was obtained at high temperature and low water content. Up to 10% of the sucrose was hydrolysed. Screw speed did not influence sucrose hydrolysis.

Results on wheat indicated that in addition to temperature, an increase in screw speed had a detrimental effect on lysine retention (Asp and Björck, 1981) (Creusot-Loire BC 45, $t_m = 156\text{--}171^\circ\text{C}$, $\text{H}_2\text{O} = 15\%$, 200 g min^{-1} , 100–200 rpm).

During extrusion cooking of lysine-fortified rice, lysine retention ranged from 89 to 97% (Tsao, 1976) (Brabender plasticorder Model PL-V500, $t_m = 133\text{--}181^\circ\text{C}$, $\text{H}_2\text{O} = 15\text{ or }20\%$, 75 or 100 rpm, c.r. = 1:1 or 3:1, die $\phi = 0.159\text{ or }0.318\text{ cm}$, pressure = 1–2.7 MPa). An increase in process temperature, screw compression ratio and screw speed

increased lysine degradation, while an increase in moisture content and die diameter had the opposite effect. At high torque and pressure, the lysine loss exceeded values predicted for thermal degradation. Disruption of starch molecules was suggested as an explanation.

In a study by Björck *et al.* (1983) the FDNB method for determination of available lysine (Booth, 1971) was compared with a biological assay with rats. The product, a protein-enriched biscuit, contained 20% sucrose (Creusot-Loire BC 45, $t_m = 170\text{--}210^\circ\text{C}$, $\text{H}_2\text{O} = 13$ or 18%, 80 rpm). At 170°C and 13% moisture, the measured loss of available lysine was similar with both methods, and also similar to the loss of total lysine, about 11%. This loss is close to that reported during conventional bread baking (Rosenburg and Rohdenburg, 1951). At higher temperatures the biological assay indicated a greater loss (50%) than the FDNB method (37%), mainly due to a decrease in protein digestibility. A higher water content increased lysine retention.

In conclusion, lysine retention during extrusion cooking is strongly influenced by process temperature. All available data indicate that a higher feed moisture content significantly improves retention. This could be a secondary effect since water, by functioning as a lubricant, reduces friction. However, the beneficial effect of moisture has been demonstrated also in studies where mass temperature in the compression chamber was kept constant. According to Noguchi *et al.* (1982), improved lysine retention at a higher water content could be explained by the law of mass action, as water is produced in the reversible phase of the Maillard reaction. However, an increase in moisture content from 15 to 20% under extrusion-like conditions, did not have any effect on the rate constant of lysine degradation (Tsao *et al.*, 1978). In a model system at low temperatures, the rate of the browning reaction is maximal at intermediate water activities. At a low water activity diffusion becomes rate limiting, and at high water activities the reaction rate decreases due to dilution of reacting substances (Eichner, 1975). It is not possible to discuss water activity inside an extruder, as it is not possible to measure it. During intense mixing, diffusion resistance at a low water content could be circumvented. An increased water content, by reducing shear (Andersson *et al.*, 1970), could reduce hydrolysis of starch and thus have a beneficial effect on protein nutritional value.

The effect of screw speed is difficult to interpret. An increase in screw speed could either reduce residence time (compression screw: Olkku *et al.*, 1980; Bartels *et al.*, 1982) or have no effect (counter-

screw element: Noguchi *et al.*, 1982). In spite of this, Tsao (1976) and Asp and Björck (1981) found a correlation between lysine destruction and screw speed. This could be an indirect effect of starch hydrolysis occurring at higher shear. Kervinen *et al.* (1981) describe the influence of increased screw speed as a combined effect. Increased shear leads to more severe conditions, but on the other hand the corresponding decrease in residence time reduces the duration of heat treatment. In cases where only barrel temperature is measured, an increased shear may affect the actual product temperature.

Under carefully selected process conditions good retention of available lysine can be obtained. A summary of the effects of different extrusion variables is given in Table 1.

Effects on other amino acids

As judged from amino acid analysis, lysine was found to be most seriously affected by a decrease in moisture content during extrusion cooking of maize grits by Köhler (1981) (Brabender DN 20, $t_m = 181-187^\circ\text{C}$, $\text{H}_2\text{O} = 12-25\%$, torque = 35-79 Nm). Below 14.5% water there was also a decrease in cystine. Only minor losses were found in other amino acids. However, biological evaluation revealed a decrease in availability of aspartic acid, tyrosine and arginine with decreasing moisture content.

With increasing energy input to the extruder a significant reduction in availability of several amino acids was found by Köhler. Although the loss of available lysine was most prominent (30%), the loss of available arginine (21%), histidine (15%), aspartic acid (14%) and serine (13%) was statistically significant (Fa Werner & Pfeleiderer Type C 37 SA/20D, $t_m = 135-160^\circ\text{C}$, 150 or 200 rpm).

Extrusion cooking of a cereal blend resulted in a considerable loss of arginine and to a lesser extent also of histidine (Beaufrand *et al.*, 1978) (Creusot-Loire BC 45, $t_m = 170^\circ\text{C}$, $\text{H}_2\text{O} = 10\%$, 40 rpm). In that study the loss of arginine was comparable to the decrease in total lysine. Pongor and Matrai (1976) measured the loss of available methionine and lysine during extrusion cooking of soy beans (Brady 400, $t_b = 127-154^\circ\text{C}$, $\text{H}_2\text{O} = 14\%$, $T_r = 20$ s). Lysine and methionine availability was not affected below 149°C . At the highest temperature lysine showed the greatest loss (31%), although a 13% decrease in methionine was noted.

Free amino acids are much more sensitive to damage during extrusion cooking than those in proteins. Maga and Sizer (1979) measured

TABLE 1

Extrusion cooking variable	Effects on nutrients					
	Protein	Starch		Vitamin retention		
		Gelatiniza- tion	Dextriniza- tion	Thiamin	Riboflavin	Ascorbic acid
Available lysine						
Temperature	-	+	+	-0	+0	-
Moisture	+	+	+	+	-0	-**
Screw speed	-0	-		-0	-	-
Screw compression ratio	-			0	-	-
Dams in screws			+			
Die diameter	+	-		+0	0	+
Torque and pressure	-		+			
Pressure						
Energy input	-					0

+, Increase; -, decrease; 0, no effect.

*, At high temperature, **, at high temperature, more is retained at low moisture.

the reactivity of naturally-occurring free amino acids in potato flakes (Brabender plasticorder Model PM-V500, $t_b = 70\text{--}160^\circ\text{C}$, $\text{H}_2\text{O} = 48\%$, 100 rpm). Phenylalanine, tyrosine, serine, isoleucine and lysine decreased considerably even at 70°C . At 160°C , the total loss of amino acids was 89%. Potato flakes extruded at 100 and 130°C contained higher levels of free amino acids than the product processed at 70°C . This is probably due to some hydrolysis of protein at elevated temperatures.

Texturization

One application of extruders is for texturization of defatted vegetable proteins (Smith, 1975; Cabrera *et al.*, 1979). A meat-like texture is obtained and the products can be used as meat extenders in, for instance, chili con carné, sausage and ground beef.

The mechanism of texturization is not fully understood. From a nutritional point of view it is important to study whether texturization involves formation of bonds that are more resistant to digestive enzymes. Burgess and Stanley (1976) describe texturization of soy flour as a rupturing of protein bonding and a subsequent reaggregation by intermolecular peptide bonds. The presence of hydrophobic interactions and hydrogen and disulphide bonds are also suggested. According to Cumming *et al.* (1973), during thermoplastic extrusion of soy protein the water-soluble protein breaks into subunits and becomes redistributed and/or insoluble. Jeunink and Cheftel (1979) found no significant cross-linking after thermoplastic extrusion of soybeans and fieldbeans. They interpreted the texturization as an effect of new hydrogen and disulphide bonds. This should not impair the susceptibility to enzymic proteolysis. No significant formation of lysinoalanine or lanthionine occurred.

Available data on textured vegetable proteins do not indicate any adverse effect on protein digestibility or quality. Sautier and Camus (1976) performed nitrogen balance studies in malnourished adults. Texturized maize semolina/broadbean blends were compared with a meat-based diet. No significant difference in faecal nitrogen excretion or nitrogen balance was seen.

Textured products from leguminous seeds have to be washed to reduce the content of indigestible carbohydrates such as raffinose, stachyose and other α -galactosides (Smith, 1975).

The availability of trace elements, e.g. Zn, is lower in vegetable foods than in foods of animal origin (Davies and Reid, 1979). With an increasing use of vegetable meat extenders in school meal programmes (Harper, 1979), the availability of trace elements in these products should be evaluated.

LIPIDS

During processing the nutritional value of lipids might be affected through different mechanisms such as oxidation, *cis-trans* isomerization or hydrogenation.

A decrease in fat content of extruded products has been reported by several authors. Fabriani *et al.* (1968) interpreted the decrease in extractable-fat content of extruded pasta products as a result of complexation and/or shear. As compared to other low-moisture heat treatments (pelleting, popping, flaking), extrusion cooking significantly reduced the extractable-fat content of wheat and maize (Delort-Laval and Mercier, 1976). Only 40–55% of the lipids present in the raw materials could be extracted with ethyl ether after extrusion ($t_b = 120^\circ\text{C}$, $\text{H}_2\text{O} = 25\text{--}28\%$). Nierle *et al.* (1980) also found a decrease in extractable-fat content in wheat and maize. The average fat recovery in extruded wheat was 40% and in maize only 20%. The yield was not increased with any of the organic solvents used (Creusot-Loire BC 45, $t_b = 120\text{--}180^\circ\text{C}$, $\text{H}_2\text{O} = 12\text{--}18\%$, 110 rpm).

Monoglycerides and free fatty acids form complexes with amylose during extrusion cooking (Mercier, 1980), and thus may become more difficult to extract with organic solvents. Other possible explanations of a decrease in fat content are steam distillation or thermal degradation. However, according to Nielsen (1976), the conditions during HTST extrusion cooking should not cause thermal destruction of lipids. Maga (1978) reported a decrease in the fat content of maize/soy mixtures with increasing extrusion temperature. The method of fat analysis included hydrolysis prior to extraction, and would thus rule out artefacts due to insufficient extraction from extruded materials. The decrease in total fat ranged from 0 to 15%. With increasing extrusion temperature there was a decrease in the ratio of unsaturated to saturated fatty acids. A small increase was also noted in *trans* fatty acids.

Formation of amylose-lipid complexes should not impair the utilization of the fat. Studies on rats with amylose-lysolecithin complexes indicated almost complete digestion (Holm *et al.*, 1983).

A decrease in extractable lipids has also been observed with other processes. Over half of the wheat flour free lipids were bound during dough mixing, and over three-quarters were no longer extractable from bread with petroleum ether (Chung *et al.*, 1981). Binding to starch and/or protein is suggested.

According to Eichner (1975) heating at low moisture should provide better stability to oxidation during storage due to formation of anti-oxidative Maillard compounds. However, the stability of full-fat soy flour decreased with increasing extrusion temperature, moisture content and residence time. Increased autoxidation as well as breakdown of natural antioxidants is suggested (Mustakas *et al.*, 1970).

CARBOHYDRATES

Low molecular weight carbohydrates

Hydrolysis of sucrose has been reported by several authors (Chiang and Johnson, 1977; Noguchi *et al.*, 1982).

According to Mercier and Feillet (1975), extrusion cooking of maize grits resulted in a reduction in ethanol-soluble carbohydrates. Fructose, glucose, sucrose and raffinose decreased as judged from paper chromatography (Creusot-Loire BC 45, $t_b = 70-250^\circ\text{C}$, $\text{H}_2\text{O} = 11-29\%$). Andersson *et al.* (1981) also found a decrease in sucrose, fructose and glucose (Creusot-Loire BC 45, $t_m = 142-155^\circ\text{C}$, $\text{H}_2\text{O} = 8-9\%$, 400 g min^{-1} , 150 or 200 rpm). The sugar content of a starch/gluten/wheat bran mixture decreased by 70-80%. Exclusion of gluten resulted in a higher recovery of fructose and glucose. It was suggested that the loss of these sugars was due to the Maillard reaction or possibly to incomplete extraction of sugars from processed materials.

Starch

The digestion of starch begins in the mouth by salivary α -amylase. Further hydrolysis takes place in the small intestine by α -amylase from the pancreas. Resulting oligosaccharides are split to glucose by α -glucosidases, i.e. enzymes with maltase and isomaltase activities, in the brush-border membrane of the intestinal mucosa. A recent study also suggests the presence of α -glucosidase in the pancreatic juice (Marshall and

Whelan, 1979). These enzymes digest pure starch very rapidly *in vivo*, rendering the absorption at the brushborder level the rate limiting step (Dahlqvist and Borgström, 1961; Wahlqvist *et al.*, 1978). However, as components in foods, different starches are digested and absorbed at different rates (Crapo *et al.*, 1977; Jenkins *et al.*, 1982).

Cooking and gelatinization of starch are known to increase the susceptibility to amylase hydrolysis, mainly due to hydration of starch granules and partial solubilization of starch molecules. Potato starch is readily solubilized during boiling, whereas cereal starches still contain fairly large amounts of undispersed granules even after several hours at 100°C (Banks and Greenwood, 1975). Apart from affecting the substrate, thermal treatment inactivates α -amylase inhibitors present in raw cereals (Granum, 1979).

Gelatinization of starch during extrusion cooking

Gelatinization of wheat flour was studied by Chiang and Johnson (1977) (Brabender Model 2503, $t_b = 65\text{--}110^\circ\text{C}$, $\text{H}_2\text{O} = 18\text{--}27\%$, 60–140 rpm, die $\phi = 0.313\text{--}0.938$ cm). Increased extrusion temperature resulted in a higher degree of gelatinization. An increase in feed moisture content had a positive effect at high temperatures. Increase in screw speed and die diameter reduced gelatinization. Complete gelatinization was obtained at 110°C and 24 or 27% moisture. According to Lawton *et al.* (1972), maximum gelatinization of maize starch occurred at a high moisture content and low temperature or vice versa (Brabender 3/4 in laboratory extruder, $t_b = 90\text{--}150^\circ\text{C}$, $\text{H}_2\text{O} = 27\text{--}29\%$, 20 or 40 rpm).

Rabe *et al.* (1980) found almost complete gelatinization in maize, maize starch and wheat starch (Creusot-Loire BC 45, $t_b = 150\text{--}170^\circ\text{C}$, $\text{H}_2\text{O} = 16\text{--}22\%$). When the temperature was raised above 135°C, no intact starch granules remained (Mercier, 1980) (Creusot-Loire BC 45, $t_b = 70\text{--}250^\circ\text{C}$, $\text{H}_2\text{O} = 22\%$). In another study on wheat in a single-screw extruder, complete destruction of starch granules was obtained above 125°C (Kim and Rottier, 1980).

Andersson *et al.* (1970) compared roller cooking, steam cooking and extrusion cooking for gelatinization of maize grits. At a comparable water absorption index suitable for production of weaning food, extrusion cooking gave the highest water solubility index.

Available data thus indicate that in spite of the low water content during extrusion, complete gelatinization is usually possible.

Dextrinization

Heat treatment of starch suspensions at high temperature and pressure may result in degradation of starch to lower molecular weight carbohydrates (Lorenz and Johnsson, 1972), i.e. dextrins.

During twin-screw extrusion cooking of potato starch, hydrolysis of $\alpha(1-4)$ glycoside bonds in amylose has been demonstrated (Mercier, 1977). The maximum yield of oligosaccharides was obtained at 190°C (Creusot-Loire BC 45, $t_b = 75-190^\circ\text{C}$, $\text{H}_2\text{O} = 23\%$). Extrusion cooking therefore provides an alternative to enzymic methods for production of linear maltodextrins for infant foods. In contrast with potato starch, cereal starches were solubilized without formation of maltodextrins (Mercier and Feillet, 1975) (Creusot-Loire BC 45, $t_b = 70-250^\circ\text{C}$, $\text{H}_2\text{O} = 11-29\%$). However, results on cereal starches are contradictory. According to Wells (1976) some hydrolysis of cereal flours occurs. Extrusion cooking of a maize/soy blend was also accompanied by some dextrinization (Sahagun and Harper, 1980). Increasing the number of internal screw restrictions, inducing higher shear, resulted in an increase in reducing groups due to hydrolysis of starch (Brady Model 206, $t_b = 149-171^\circ\text{C}$, 500-1000 rpm).

The effect of different extrusion variables on gelatinization and dextrinization is summarized in Table 1.

Amylose-lipid complexes

Formation of a complex between amylose and fatty acids has been observed in X-ray spectra (Mercier, 1980) (Creusot-Loire BC 45, $t_b = 70-250^\circ\text{C}$, $\text{H}_2\text{O} = 22$ or 33%). At 22% moisture the complex appears at 135°C, whereas at 33% moisture it occurs at 70°C. This complexed amylose was resistant to α -amylase *in vitro*. With increasing amylose content (waxy maize < maize < amylon 5 < amylon 7), there was a decrease in the rate of amylolysis after extrusion. According to Larsson and Miezi (1979) complexation of amylose with monoolein rendered the amylose relatively stable to pancreatic amylase.

Formation of amylose-lipid complexes has certain technological advantages in that it reduces the stickiness of the product (Mercier *et al.*, 1980). From both nutritional and analytical points of view it is important to know whether these complexes are digested and should be looked upon as starch or not digested and looked upon as dietary fibre (Larsson and Miezi, 1979). Recent studies by Holm *et al.* (1983), showed that amylose-lysolecithin and amylose-oleic acid complexes

were less available to α -amylase *in vitro* than soluble amylose. However, with excess of amylase and prolonged incubation time, complete degradation was obtained. In the rat, amylose-lysolecithin complexes were completely digested and absorbed. The rate of absorption was only slightly lower than that of free amylose.

Availability of starch for enzymic degradation

The rate of carbohydrate absorption from the intestine is of interest in relation to obesity and diabetes. According to Heaton (1980) the insulinogenicity of a food item increases as a result of cooking and homogenization, as well as by disruption or depletion of dietary fibre.

Particle size appears to influence the rate of α -amylase digestion *in vitro*. Boiled, fine-ground, whole grain wheat flour was more available to α -amylase than boiled, coarse-ground flour (Greenberg, 1976). An increase in starch availability has been demonstrated also *in vivo* for milled versus whole rice (O'Dea *et al.*, 1981). Gelatinization in combination with a thorough mixing, such as occurs during extrusion cooking, could therefore be expected to render the starch readily available to amylase.

The susceptibility of cereal starches to α -amylase hydrolysis increased with increasing extrusion temperature. Milling of the extrudates significantly enhanced penetration of amylase. However, after extrusion at a high temperature (225°C), the accessibility to α -amylase was not improved further by milling (Mercier, 1980) (Creusot-Loire BC 45, $t_b = 135^\circ\text{C}$ or 225°C , $\text{H}_2\text{O} = 22\%$). Increasing the energy input to the extruder increased the availability to amyloglucosidase (Köhler, 1981). The initial rate and final degree of starch hydrolysis were lower in extruded maize based blends than after autoclaving in water for 1 h at 130°C . However, maize grits processed in a twin-screw cooker extruder at high screw speed were almost as available as the autoclaved control. Compared to other low-moisture heat treatments, such as flaking, popping and pelleting, extrusion cooking led to the highest rate of initial amylolysis in wheat and maize (Delort-Laval and Mercier, 1976).

In a study by Asp and Björck (1982) the availability of starch in cooker-extruded wheat samples was compared with boiling for 20 min in water (Creusot-Loire BC 45, $t_m = 161\text{--}171^\circ\text{C}$, $\text{H}_2\text{O} = 15$ or 20% , 200 or 350 g min^{-1} , $100\text{--}200\text{ rpm}$). Suspensions of extruded wheat samples were more available to salivary amylase *in vitro* than boiled controls. Autoclaving (20 min, 125°C) increased the availability to the

same level as extrusion cooking did. The glucose peak response in rats was higher for wheat samples cooker-extruded under the most severe conditions than for those boiled. The insulin response was also greater. In contrast, mild cooker-extrusion conditions gave responses similar to those found with boiling. The wheat flour cooker-extruded under severe conditions ($t_m = 171^\circ\text{C}$, $\text{H}_2\text{O} = 15\%$, 200 g min^{-1} , 200 rpm) caused diarrhoea in rats during feeding experiments. This may be due to formation of indigestible carbohydrates under extreme process conditions.

Dietary fibre

Dietary fibre includes non-starch polysaccharides and lignin that are resistant to digestive enzymes. In the large intestine, however, fermentation occurs due to bacterial enzymes. The availability to bacterial degradation varies with the type of fibre (Nyman and Asp, 1982). Cereal fibre is important quantitatively, and its good faecal bulking effect is mainly due to a high resistance to fermentation.

Very few studies have been made of the effect of extrusion cooking or other processes on dietary fibre. Degradation may occur in processes that involve shear. It is known that during dry ball milling, glycoside bonds in cellulose may be broken (Theander, 1981). According to Thomas and Elchazly (1976) degradation of fibre in the colon is inversely proportional to the size of the fibre particles. Disruption and homogenization of bran particles by the intense mechanical treatment during extrusion cooking could therefore render dietary fibre more available to fermentation.

Analysis of extruded wheat flour (performed according to Asp *et al.*, 1983) indicated an increase in water-soluble fibre at the expense of water-insoluble. The redistribution was more pronounced in wheat flour than in whole-grain wheat flour. In raw wheat flour 40% of the fibre was soluble as against 70% after extrusion cooking (Asp and Björck, 1982) (Creusot-Loire BC 45, $t_m = 161\text{--}171^\circ\text{C}$, $\text{H}_2\text{O} = 15$ or 20% , 200 g min^{-1} , $100\text{--}200\text{ rpm}$). Fibre balance experiments performed on rats did not indicate an increased fermentability of fibre in extruded whole-grain wheat flour. However, with extruded wheat flour there was an increase in fermentation compared with the raw material, even with mild extrusion conditions.

In a collaborative study organized by Varo *et al.* (1983) the dietary fibre content of cooker-extruded wheat and whole grain wheat flours was compared with the raw materials. Different analytical methods were evaluated. With some methods, there was an increase in water-soluble fibre after extrusion. However, when results from all methods were combined, no significant difference in fibre content or distribution was found (Creusot-Loire BC 45, $t_m = 161-180^\circ\text{C}$, $\text{H}_2\text{O} = 15\%$, 200 or 350 g min^{-1} , 150 or 200 rpm). Obviously the solubility of dietary fibre is highly dependent on the method of determination.

Further studies are needed to establish whether solubilization of fibre, as measured by an analytical procedure, correlates with an increase in fermentation of the fibre in the large intestine.

Another well-documented effect of dietary fibre is improved glucose tolerance (reduced rate of rise of blood sugar following a meal). This effect is most pronounced with gelling fibres like pectins or guar gum (Jenkins, 1979), but addition of wheat bran to the diet during a longer period has been shown to improve glucose tolerance (Brodrick and Humphreys, 1976). According to Nygren *et al.* (1982), cooker-extruded wheat bran retained its antidiabetic effect on rats with experimental diabetes.

VITAMINS

The fate of B-vitamins during extrusion cooking is important, cereals being a good source of these vitamins. Precooked cereal-based weaning foods are often fortified with vitamins. Occasionally, to reduce contamination with microorganisms, vitamin fortification is made prior to extrusion, and thus the retention of total vitamin content needs to be considered.

Vitamins can be damaged by mechanisms such as heat and oxidation. In addition, conventional boiling in water can lead to considerable loss of water-soluble vitamins due to leaching (Archer and Tannenbaum, 1979).

B-vitamins

Vitamin retention during extrusion cooking has been studied most in the case of thiamin and riboflavin. It is mainly for these vitamins that correlations have been made with process variables. During conventional

processing, thiamin is most labile to heat while riboflavin is comparatively thermostable. Thiamin, riboflavin and niacin were unaffected during extrusion cooking of full-fat soy flour (Mustakas *et al.*, 1964) (Wenger, $t_b = 139$ or 153°C , $\text{H}_2\text{O} = 9$ or 12% , $T_r = 1$ min). In another study on FFSF, process conditions were varied over a wider range (Mustakas *et al.*, 1970) (pilot-plant plastics extruder, $t_b = 139$ – 167°C , $\text{H}_2\text{O} = 15$ – 30% , $T_r = 0.5$ – 2 min). The average thiamin recovery was 79%. No significant correlation was found with process conditions.

The stability of thiamin and riboflavin in maize grits was studied by Beetner *et al.* (1974) (Brabender plasticorder, $t_b = 167$ – 211°C , $\text{H}_2\text{O} = 13$ – 16% , 75–125 rpm). The average retention was 54% thiamin and 92% riboflavin. An increased degradation was seen for thiamin with increasing temperature and screw speed. Moisture content did not have any effect. Riboflavin degradation increased with increasing moisture content and screw speed. The effect of temperature on riboflavin was not significant. Thus for both vitamins an increase in screw speed had a detrimental effect.

Under extrusion conditions producing a realistic breakfast cereal from triticale the loss of thiamin and riboflavin was substantial – 90% of the thiamin and 50% of the riboflavin (Beetner *et al.*, 1976) (Brabender plasticorder, $t_b = 194$ – 250°C , $\text{H}_2\text{O} = 15$ – 25% , die $\phi = 0.156$ or 0.313 cm). Thiamin loss decreased with increasing die diameter and decreasing temperature. The loss of riboflavin correlated only with barrel temperature, an increased temperature resulting in a higher retention. This apparently beneficial effect of heat may be due to a decrease in shear at higher temperatures. An improved stability of riboflavin at higher temperature has also been reported by Harper *et al.* (1977). At 154°C the retention of riboflavin in an extruded maize/soy blend was 65–72%, whereas 95–100% was retained at 171°C (Insta-Pro, $t_b = 154$ or 171°C).

In a study on extruded potato flakes, Maga and Cohen (1978) found only minor losses of thiamin (Brabender plasticorder Model PL-V500, $t_b = 135$ – 177°C , $\text{H}_2\text{O} = 20\%$, 40–200 rpm, screw c.r. = 1/1 or 1/3, die $\phi = 0.156$ or 0.313 cm). Although the shear conditions were varied over a wide range, thiamin loss did not exceed 15%.

The importance of moisture content on thiamin stability was demonstrated by Maga and Sizer (1978) (Brabender plasticorder Model PL-V500, $t_b = 70$ – 160°C , $\text{H}_2\text{O} = 25$ – 59% , 100 rpm, $T_r = 0.5$ – 2.25 min).

Under the conditions used thiamin retention in extruded potato flakes ranged from 22 to 97%. The retention was poor at low moisture contents. At low moisture, product output was reduced, thus increasing residence time and internal pressure. According to Beetner (1978) hydrostatic pressure *per se* has no influence on B-vitamin degradation. A similar decrease in thiamin stability at lower water contents was obtained in wheat flour by Andersson and Hedlund (1982). In the range of conditions used, feed moisture content was a more crucial factor than temperature (Creusot-Loire BC 45, $t_m = 105\text{--}200^\circ\text{C}$, $\text{H}_2\text{O} = 14\text{--}34\%$, $250\text{--}750\text{ g min}^{-1}$). At 14% moisture there was a substantial loss of thiamin (60–90%). In contrast, at a high moisture content thiamin was only slightly affected even at 200°C . In general, a higher feed rate improved retention. Niacin and riboflavin showed a high stability under the conditions of the test.

During extrusion cooking of maize/soy blends the average loss of thiamin, riboflavin, vitamin B₆ and folic acid was only about 10% (Brady extruder, $t_b = 171^\circ\text{C}$). However, under similar extrusion conditions in another study, significantly higher thiamin losses of 25–50% occurred (Brady extruder, $t_b = 163^\circ\text{C}$) (Jansen, 1979). In view of this variability in thiamin retention, vitamin fortification after extrusion cooking is preferable. Niacin stability was slightly higher during extrusion cooking of a maize/soy/groundnut blend than on boiling for 2 min (de Muelenaere and Buzzard, 1969) (Sprout Waldron).

Thus, quite divergent results are reported concerning the effect of different processing variables on thiamin and riboflavin. From the experiments by Beetner *et al.* (1974, 1976) it has been concluded that shear has a detrimental effect on thiamin and riboflavin (Harper, 1979). However, it is not known why riboflavin degradation is more pronounced at higher water contents. Further, no significant effect of die diameter was observed on riboflavin retention. An increase in moisture content or die diameter should reduce shear. In these studies thiamin losses increased with increasing temperature and shear energy. In contrast, at a slightly lower temperature range, Maga and Cohen (1978) did not find any effect of mechanical shear on thiamin retention.

In conclusion, riboflavin appears to be retained well during extrusion while thiamin retention appears to be highly dependent on process conditions. As judged from the few available data, niacin, pyridoxin and folic acid appear to be comparatively stable during extrusion cooking.

Ascorbic acid

During conventional cooking in water, large losses of vitamin C may result from a combination of oxidation and leaching. Pressure cooking is less detrimental, with about 80% vitamin C retention (Davidson *et al.*, 1979).

Boiling for 2 min caused 79% destruction of added vitamin C in a maize/soy/groundnut mixture. Extrusion cooking was much less detrimental, only 33% being destroyed (de Muelenaere and Buzzard, 1969) (Sprout Waldron). Processing of a fortified maize/soy blend in a low-cost extrusion cooker retained 80% or more vitamin C (Lorenz *et al.*, 1980) (Brady extruder, 171°C). In this study an interesting phenomenon was observed during storage (31°C, 48% RH). When vitamin C was added before extrusion, great losses occurred (57–66%). In contrast, when vitamin C was added after extrusion, the loss during storage was similar to that of thiamin (14–20%). Further, vitamin C stability during storage of extruded cereal/soy blends was much higher than in the corresponding raw material. It is suggested that the beneficial effect of extrusion cooking is related to a reduced water activity (Harper and Jansen, 1981). Thus, the best storage stability is obtained in the processed product where vitamin C fortification has been done after processing.

Maga and Sizer (1978) studied the retention of vitamin C in potato flakes (Brabender plasticorder Model PL-V500, $t_b = 70$ – 160°C , $\text{H}_2\text{O} = 25$ – 59% , 100 rpm). Vitamin C losses ranged from 14 to 68%. At high process temperatures, more vitamin C was retained at the lowest moisture content. No correlation was found with pressure. Andersson and Hedlund (1982) also noted a greater stability of ascorbic acid in low moisture wheat flour at elevated temperatures (Creusot-Loire BC 45, 105 – 200°C , $\text{H}_2\text{O} = 14$ – 34% , 250 – 750 g min^{-1}). The beneficial effect of low moisture is in agreement with results from model systems (Lee and Labuza, 1975). Another interesting aspect is the influence of iron addition through screw wear (Maga and Sizer, 1978). Ferric ions are known to catalyze the destruction of vitamin C. The iron content of extruded products increased with increasing temperature (3.4 mg Fe per 100 g at 70°C ; 13.2 mg Fe per 100 g at 160°C). It is suggested that this increase in iron content may contribute to the increased vitamin C destruction at higher temperatures.

The effect of screw speed, compression ratio and die diameter on vitamin C retention was studied by Maga and Cohen (1978) (Brabender

plasticorder Model PL-V500, $t_b = 135\text{--}177^\circ\text{C}$, $\text{H}_2\text{O} = 20\%$, 40–200 rpm, screw c.r. = 1/1 or 1/3, die $\phi = 0.156$ or 0.313 cm). The losses of vitamin C in cooker-extruded potato flakes ranged from 9 to 57%. Less destruction occurred with the larger die and with the 1/1 screw c.r. An increased screw speed and temperature resulted in greater losses. It should be noted that the snack product that was most acceptable from a sensory point of view had the lowest content of vitamin C.

Fat-soluble vitamins

Compared to boiling for 2 min, extrusion cooking of a maize/soy/groundnut blend resulted in greater losses of carotene. Only 25% was destroyed during boiling versus 53% during extrusion cooking (de Muelenaere and Buzzard, 1969) (Sprout Waldron).

The stability of different compounds with vitamin A activity was studied by Lee *et al.* (1978). White maize meal was fortified prior to extrusion with β -carotene, retinol, retinyl acetate or retinyl palmitate (Wenger X5, $t_b = 130^\circ\text{C}$, 700–1000 rpm). All vitamin A forms were more stable at higher screw speeds, probably due to a shorter residence time. Retinyl acetate showed the highest stability. The retention of retinol was 83–94% and that of the palmitate 52–91%, respectively. However, only about 26% β -carotene could be detected in the extruded product. The low recovery of β -carotene could be due to insufficient extraction, as β -carotene was more tightly bound after extrusion. In contrast, improved extractability of fat-soluble vitamins has been reported. The vitamin A levels in extruded maize/soy blends were consistently 30–60% higher than in the raw material. It is suggested that extrusion cooking improves extractability of vitamin A, or that coloured compounds formed during processing interfere with the colorimetric assay (Harper *et al.*, 1977) (Insta Pro, $t_b = 154$ or 171°C). Vitamin E retention during extrusion cooking of a similar product also exceeded 100%. Vitamin A retention ranged from 87 to 104% (Jansen, 1979) (Brady, 163°C).

At 2 min residence time, tocopherol losses in full-fat soy flour were less than 15% (Mustakas *et al.*, 1970). No losses could be detected at shorter residence times (pilot-plant plastics extruder, $t_b = 139\text{--}167^\circ\text{C}$, $\text{H}_2\text{O} = 15\text{--}30\%$, $T_r = 0.5\text{--}2$ min).

Due to difficulties in the analytical procedures, the stability of fat-soluble vitamins needs further evaluation.

A summary of the influence of different extrusion variables on vitamin retention is presented in Table 1. As already pointed out by Maga and Sizer (1978), conditions leading to an improved retention of one vitamin may lead to increased degradation of another.

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REFERENCES

- Adrian, J. (1974). Nutritional and physiological consequences of the Maillard reaction. *World Review of Nutrition and Dietetics*, **19**, 71-122.
- Aguilera, J. M. and Kosikowski, F. V. (1978). Extrusion and roll-cooking of corn-soy-whey mixtures. *J. Food Sci.*, **43**, 225-30.
- Andersson, Y. and Hedlund, B. (1982). The Swedish Food Institute, SIK, Gothenburg (private communication).
- Andersson, R. A., Conway, H. F. and Peplinski, A. J. (1970). Gelatinization of corn grits by roll cooking, extrusion cooking and steaming. *Die Stärke*, **22** (4), 130-4.
- Andersson, Y., Hedlund, B., Jonsson, L. and Svensson, S. (1981). Extrusion cooking of a high-fiber cereal product with crispbread character. *Cereal Chem.*, **58** (5), 370-4.
- Archer, M. C. and Tannenbaum, S. R. (1979). Vitamins. In: *Nutritional and Safety Aspects of Food Processing*, ed. S. R. Tannenbaum, Marcel Dekker, New York and Basel, pp. 47-95.
- Asp, N.-G. and Björck, I. (1981). Influence of extrusion cooking on the nutritional value. *Nordforsk-SIK-SNF Seminar: Extrusion Cooking of Food*, Gothenburg.
- Asp, N.-G. and Björck, I. (1982). Effects of extrusion cooking on the nutritional value. *Proc. 7th World Cereal and Bread Congress*, Prague.
- Asp, N.-G., Johansson, C.-G., Hallmer, H. and Siljeström, M. (1983). *J. Agric. Food Chem.* (in press).
- Banks, W. and Greenwood, C. T. (1975). *Starch and Its Components*, Edinburgh University Press, p. 9.
- Bartels, P. V., Janssen, L. P. B. M. and van Zuilichem, D. I. (1982). Residence time distribution in a single screw cooking extruder. *Seminar ZDS. Koch und Extrudier Techniken*, Solingen-Gräfrath.
- Beaufrand, M. J., de la Guérivière, J. F., Monnier, C. and Poullain, B. (1978).

- Influence du procédé de cuisson extrusion sur la disponibilité des protéines. *Ann. Nutr. Aliment.*, **32**, 353-64.
- Beetner, G. (1978). *Processing effects on vitamin retention*. Dissertation. Colorado State University, Fort Collins.
- Beetner, G., Tsao, T., Frey, A. and Harper, J. (1974). Degradation of thiamine and riboflavin during extrusion processing. *J. Food Sci.*, **39**, 207-8.
- Beetner, G., Tsao, T., Frey, A. and Lorenz, K. (1976). Stability of thiamine and riboflavin during extrusion processing of triticale. *J. Milk Food Technol.*, **39** (4), 244-5.
- Björck, I., Noguchi, A., Asp, N.-G., Cheftel, J. C. and Dahlqvist, A. (1983). Protein nutritional value of a biscuit processed by extrusion cooking – effects on available lysine. *J. Food Sci. Agric.* (in press).
- Bookwalter, G. N., Mustakas, G. C., Kwolek, W. F., McGhee, J. E. and Albrecht, W. J. (1971a). Full-fat soy flour extrusion cooked-properties and food uses. *J. Food Sci.*, **36**, 5-9.
- Bookwalter, G. N., Kwolek, W. F., Black, L. T. and Griffin, E. L. (1971b). Corn meal soy flour blends – characteristics and food applications. *J. Food Sci.*, **36**, 1026-32.
- Booth, V. H. (1971). Problems in the determination of FDNB-available lysine. *J. Sci. Food Agric.*, **22** (12), 658-66.
- Brodribb, A. J. M. and Humphreys, D. M. (1976). Diverticular disease: three studies. Part III: Metabolic effects of bran. *British Med. J.*, **1**, 428-30.
- Burgess, L. D. and Stanley, D. W. (1976). Research note – a possible mechanism for thermal texturization of soy bean protein. *Can. Inst. Food Sci. Technol. J.*, **9** (4), 228-31.
- Cabrera, J., Zapata, L. E., de Buckle, T. S., Ben-Gera, I., de Sandoval, A. M. and Shomer, I. (1979). Production of textured vegetable protein from cottonseed flours. *J. Food Sci.*, **44**, 826-30.
- Chiang, B.-Y. and Johnson, J. A. (1977). Gelatinization of starch in extruded products. *Cereal Chem.*, **54** (3), 436-43.
- Chung, O. K., Tsen, C. C. and Robinson, R. J. (1981). Functional properties of surfactants in breadmaking. III. Effects of surfactants and soy flour on lipid binding in breads. *Cereal Chem.*, **58** (3), 220-6.
- Crapo, P. A., Reaven, G. and Olefsky, J. (1977). Postprandial plasma-glucose and insulin responses to different complex carbohydrates. *Diabetes*, **26** (12), 1178-83.
- Cumming, D. B., Stanley, D. W. and De Man, J. M. (1973). Fate of water soluble soy protein during thermoplastic extrusion. *J. Food Sci.*, **38**, 320.
- Dahlqvist, A. and Borgström, B. (1961). Digestion and absorption of disaccharides in man. *Biochem. J.*, **81**, 411-18.
- Davidson, S., Passmore, R., Brock, J. F. and Truswell, A. S. (1979). Losses of food

- and nutrients in food processing. In: *Human Nutrition and Dietetics*, Churchill Livingstone, Edinburgh, pp. 210-13.
- Davies, N. T. and Reid, H. (1979). An evaluation of the phytate, zinc, copper, iron and manganese contents of, and availability from, soya-based textured vegetable protein, meat-substitutes or meat-extendors. *Br. J. Nutr.*, **41**, 579-89.
- Delort-Laval, J. and Mercier, C. (1976). Selection of treatments and study of their influence on the carbohydrate fraction of wheat, barley and maize. *Ann. Zoo-techn.*, **25** (1), 3-12.
- del Valle, F. R., Villanueva, H., Reyes-Govea, J., Escobedo, M., Bourges, H., Ponce, J. and Munoy, M. J. (1981). Development, evaluation and industrial production of a powdered soy-oats infant formula using a low-cost extruder. *J. Food Sci.*, **46**, 192-7.
- de Muelenaere, H. J. H. and Buzzard, J. L. (1969). Cooker extruders in service of world feeding. *Food Technol.*, **23**, 345-51.
- Eichner, K. (1975). The influence of water content on non-enzymic browning reaction in dehydrated foods and model systems and the inhibition of fat oxidation by browning intermediates. In: *Proc. of an International Symposium on Water Relations of Foods, Glasgow, 1974*, ed. R. B. Duckworth, Academic Press, London, pp. 417-34.
- Essatara, M. and Saintaurin, M.-A. (1976). Efficiency of various treatments of cereals. III - Effect of popping, extrusion and flaking on the feeding value of wheat and maize for growing rats. *Ann. Zootechn.*, **25** (1), 31-9.
- Fabriani, G., Lintas, C. and Quaglia, G. B. (1968). Chemistry of lipids in processing and technology of pasta products. *Cereal Chem.*, **45**, 454-63.
- Granum, P. E. (1979). Studies of α -amylase inhibitors in foods. *Food Chem.*, **4**, 173-8.
- Greenberg, C. J. (1976). *Studies on the fibre in human diets and its effect on the digestion and absorption of other nutrients*. Dissertation. Newnham College, Cambridge.
- Griensen, A. C. (1981). CIATECH: A summary of its work using soya to produce nutritional food at low cost. *JAOCs March*, pp. 385-7.
- Harper, J. M. (1979). Food extrusion. *Crit. Rev. Food Sci. Nutr.*, **11** (2), 155-215.
- Harper, J. M. (1981). *Extrusion of Foods*, vols I and II, CRC Press, Boca Raton, Florida.
- Harper, J. M. and Jansen, G. R. (1981). Nutritious foods produced by low-cost technology. *LEC Report 10, Colorado State University*, Fort Collins, Colorado.
- Harper, J. M., Stone, M. L., Tribelhorn, R. E., Jansen, G. R., Lorenz, K. J. and Maga, J. A. (1977). Evaluation of low-cost extrusion cookers for use in LDCs. *LEC Report 2, Colorado State University*, Fort Collins, Colorado.
- Heaton, K. W. (1980). *Medical Aspects of Dietary Fibre - Topics in Gastroenterology*, eds G. A. Spiller and R. McPherson Kay, Plenum Medical Book Comp., New York and London, pp. 223-38.

- Holm, J., Björck, I., Ostrowska, S., Eliasson, A. C., Asp, N.-G., Larsson, K. and Lundqvist, I. (1983). Digestibility of amylose lipid complexes *in vitro* and *in vivo*. *Die Stärke* (in press).
- Horan, F. E. (1966). Defatted and full-fat soy flours by conventional processes. *Proc. Int. Conf. Soybean Protein Foods*, Peoria, Illinois, USDA, ARS, **71** (35), p. 129.
- Hurrell, R. F. and Carpenter, K. J. (1977). Maillard reactions in foods. In: *Physical, Chemical and Biological Changes in Food Caused by Thermal Processing*, eds T. Høyem and O. Kvåle, Applied Science Publishers Ltd, London, pp. 168-84.
- Jansen, G. R. (1979). Nutritional aspects of the LEC program at Colorado State University. In: *Low-cost Extrusion Cookers*, eds D. E. Wilson and R. E. Tribelhorn, Colorado State University, Fort Collins, pp. 121-41.
- Jansen, G. R. and Harper, J. M. (1980). Application of low-cost extrusion cooking to weaning foods in feeding programs. Part 1. *Food and Nutr.*, **6** (1), 2-9.
- Jansen, G. R., Harper, J. M. and O'Deen, L. (1978). Nutritional evaluation of blended foods made with a low-cost extruder cooker. *J. Food Sci.*, **43**, 912-16.
- Jenkins, D. J. A. (1979). Dietary fibre, diabetes, and hyperlipidaemia. Progress and prospects. *The Lancet*, **15**, 1288-90.
- Jenkins, D. J. A., Ghafari, H., Wolever, T. M. S., Taylor, R. H., Jenkins, A. L., Barker, H. M., Fielden, H. and Bowling, A. C. (1982). Relationship between rate of digestion of foods and post-prandial glycaemia. *Diabetologia*, **22**, 450-55.
- Jeunink, J. and Cheftel, J. C. (1979). Chemical and physicochemical changes in field bean and soybean proteins texturized by extrusion. *J. Food Sci.*, **44** (5) 1322-5, 1328.
- Jokinen, J. E., Reineccius, G. A. and Thompson, D. R. (1975). Losses in available lysine during thermal processing of soy protein model systems. *IFT Annual Meeting*, Chicago, Illinois.
- Kervinen, R., Linko, P. and Olkku, J. (1981). Extrusion cooking induced changes in functional properties of wheat flour. *Nordiskt cerealistförbunds 21: a kongress, Espoo*.
- Kim, J. C. and Rottier, W. (1980). Modification of aestivum wheat semolina by extrusion. *Cereal Foods World*, **24**, 62-6.
- Köhler, F. (1981). *Veränderung der ernährungsphysiologischen und physikalischen eigenschaften von getreidemahler zeugnissen durch extrusion unter besonderer berücksichtigung proteinangereicherter produkte*. Dissertation. Institute für Lebensmittel-technologie, Berlin.
- Krogdahl, Å. and Holm, H. (1979). Proteinase inhibitorer i soyabønner. *Næringsforsk.*, **1**, 2-11.
- Larsson, K. and Miezi, Y. (1979). On the possibility of dietary fiber formation by interaction in the intestine between starch and lipids. *Die Stärke*, **31** (9), 301-2.
- Lawton, B. T., Henderson, G. A. and Derlatka, E. J. (1972). The effects of extruder variables on the gelatinization of corn starch. *Can. J. Chem. Eng.*, **50**, 168-72.

- Lea, C. H. and Hannan, R. S. (1949). The effect of activity of water, of pH and of temperature on the primary reaction between casein and glucose. *Biochim. Biophys. Acta.*, **3**, 313-25.
- Lee, S. H. and Labuza, T. P. (1975). Destruction of ascorbic acid as a function of water activity. *J. Food Sci.*, **40** (1), 370-3.
- Lee, T.-C., Chen, T., Alid, G. and Chichester, C. O. (1978). Stability of vitamin A and provitamin A in extrusion cooking processing. *AIChE Symp. Ser.*, **74** (172), 192-5.
- Linko, P., Colonna, P. and Mercier, C. (1982). High-temperature, short-time extrusion cooking. In: *Advances in Cereal Science and Technology*, vol. IV, ed. Y. Pomeranz, American Association of Cereal Chemists Inc., St. Paul, pp. 145-235.
- Lorenz, K. and Johnsson, J. A. (1972). Starch hydrolysis under high temperatures and pressures. *Cereal Chem.*, **49**, 616-28.
- Lorenz, K., Jansen, G. R. and Harper, J. (1980). Nutrient stability of full-fat soy flour and corn-soy blends produced by low-cost extrusion. *Cereal Foods World*, **25** (4), 161-2, 171-2.
- Lukoo, H. M. (1980). Extruded high-protein weaning food product. In: *Food Process Engineering*, vol. 1: Food Processing Systems, eds P. Linko, Y. Mälkki, J. Olkku and J. Larinkari, Applied Science Publishers Ltd, London, pp. 839-44.
- Maga, J. A. (1978). *Cis-trans* fatty acid ratios as influenced by product and temperature of extrusion cooking. *Lebensm. Wiss. Technol.*, **11** (4), 183-4.
- Maga, J. A. and Cohen, M. R. (1978). Effect of extrusion parameters on certain sensory, physical and nutritional properties of potato flakes. *Lebensm. Wiss. Technol.*, **11** (4), 195-7.
- Maga, J. A. and Sizer, C. E. (1978). Ascorbic acid and thiamin retention during extrusion of potato flakes. *Lebensm. Wiss. Technol.*, **11** (4), 192-4.
- Maga, J. A. and Sizer, C. E. (1979). The fate of free amino acids during the extrusion of potato flakes. *Lebensm. Wiss. Technol.*, **12** (1), 13-16.
- Marshall, J. J. and Whelan, W. J. (1979). Some aspects of dietary starch utilization in the mammalian digestive tract. *Proc. Congr. fed. Asian Oceanian. Biochemical Aspects of Nutrition*, pp. 125-30.
- Mercier, C. (1977). Effect of extrusion cooking on potato starch using a twin-screw French extruder. *Die Stärke*, **28** (2), 48-52.
- Mercier, C. (1980). Structure and digestibility alterations of cereal starches by twin-screw extrusion cooking. In: *Food Process Engineering*, vol. 1: Food Processing Systems, eds P. Linko, Y. Mälkki, J. Olkku and J. Larinkari, Applied Science Publishers Ltd, London, pp. 795-807.
- Mercier, C. and Feillet, P. (1975). Modification of carbohydrate components by extrusion cooking of cereal products. *Cereal Chem.*, **52** (3), 283-97.
- Mercier, C., Charbonniere, R., Grebaut, J. and de la Gueriviere, J. F. (1980). Formation of amylose-lipid complexes by twin-screw extrusion cooking of manioc starch. *Cereal Chem.*, **57** (1), 4-9.

- Mustakas, G. C., Griffin, E. L., Allen, L. E. and Smith, O. B. (1964). Production and nutritional evaluation of extrusion-cooked full-fat soybean flour. *J. Am. Oil Chem. Soc.*, **41**, 607-14.
- Mustakas, G. C., Albrecht, W. J., Bookwalter, G. N., McGhee, J. E., Kwolek, W. F. and Griffin, E. L. (1970). Extruder processing to improve nutritional quality, flavor and keeping quality of full-fat soy flour. *Food Technol.*, **24**, 1290-6.
- Mustakas, G. C., Albrecht, W. J., Bookwalter, G. N., Sohne, V. E. and Griffin, E. L. (1971). New process for low-cost, high-protein beverage base. *Food Technol.*, **25**, 534-40.
- Nielsen, E. (1976). Whole seed processing by extrusion cooking. *J. Am Oil Chem. Soc.*, **53**, 305-9.
- Nierle, W., Elbaya, A. W., Seiler, K., Fretzdorff, B. and Wolff, J. (1980). Veränderungen der Getreideinhaltsstoffe während der Extrusion mit einem doppel-schnecken Extruder. *Getreide Mehl. Brot.*, **34**, 73-8.
- Noguchi, A., Mosso, K., Aymard, C., Jeunink, J. and Cheftel, J. C. (1982). Maillard reactions during extrusion cooking of protein-enriched biscuits. *Lebensm. Wiss. Technol.*, **15** (2), 105-10.
- Nygren, C., Hallmans, G., Jonsson, L. and Asp, N.-G. (1982). Effects of processed rye and wheat bran on glucose metabolism. *Intern. Symp. Fibre in Human and Animal Nutrition*, Palmerston North, New Zealand, abstract no. 176.
- Nyman, M. and Asp, N.-G. (1982). Fermentation of dietary fibre components in the rat intestinal tract. *Br. J. Nutr.*, **47**, 357-66.
- O'Dea, K., Snow, P. and Nestel, P. (1981). Rate of starch hydrolysis *in vitro* as a predictor of metabolic responses to complex carbohydrate *in vivo*. *Am. J. Clin. Nutr.*, **34**, 1991-3.
- Olkku, J., Antila, J., Heikkinen, J. and Linko, P. (1980). Residence time distribution in twin-screw extruder. In: *Food Process Engineering*, vol. 1: Food Processing Systems, eds P. Linko, Y. Mälkki, J. Olkku and J. Larinkari, Applied Science Publishers Ltd, London, pp. 791-4.
- Pongor, S. and Matrai, T. (1976). Determination of available methionine and lysine in heat treated soybean samples. *Acta Alimentaria*, **5** (1), 49-55.
- Rabe, E., Seiler, K. and Gödecke, V. (1980). Analytische Bestimmungen der Stärkeverkleisterung von Extrudanten. *Zucker, Süßwaren Wirtsch.*, **33** (5), 158-62.
- Rao, M. N. and McLaughlan, J. M. (1967). Lysine and methionine availability in heated casein-glucose mixtures. *J. Assoc. Offic. Analyt. Chem.*, **50**, 704-7.
- Rosenburg, H. R. and Rohdenburg, E. L. (1951). The fortification of bread with lysine: The loss of lysine during baking. *J. Nutr.*, **45**, 593-8.
- Rubenthaler, G., Pomeranz, Y. and Finney, K. F. (1963). Effects of sugars and certain free amino acids on bread characteristics. *Cereal Chem.*, **40**, 658-65.
- Sahagun, J. F. and Harper, J. M. (1980). Effects of screw restrictions on the performance of an autogenous extruder. *J. Food Proc. Eng.*, **3**, 199-216.

- Sautier, C. and Camus, M. C. (1976). Valeur nutritionnelle et acceptabilité chez l'homme de protéines végétales texturées. *Rev. Fr. de Corps Gras*, **4**, 203-8.
- Smith, O. B. (1975). Engineering 'meat'. *Food Engineering*, **47**, 24-33.
- Theander, O. (1981). In: *The Analysis of Dietary Fibre in Food*, eds W. P. T. James and O. Theander, Marcel Dekker, New York and Basel, p. 242.
- Thomas, B. and Elchazly, M. (1976). The physiological effects and changes of the dietary fibre of wheat in the digestive tract. *Qual. Plant. Pl. Fas. Hum. Nutr. XXVI*, **1** (3), 211-26.
- Thompson, D. R., Wolf, J. C. and Reineccius, G. A. (1976). Lysine retention in food during extrusion-like processing. *Trans. ASAE*, **19** (5), 989-92.
- Tsao, T.-F. (1976). *Available lysine retention during extrusion processing*. Dissertation, Colorado State University.
- Tsao, T.-F., Frey, A. L. and Harper, J. M. (1978). Available lysine in heated fortified rice meal. *J. Food Sci.*, **43**, 1106-8.
- Tsen, C. C., Farell, E. P., Hoover, W. J. and Crowley, P. R. (1975). Extruded soy products from whole and dehulled soybeans cooked at various temperatures for bread and cookie fortifications. *Cereal Foods World*, **20** (9), 413-18.
- Varo, P., Laine, R. and Koivistoinen, P. (1982). The effect of heat treatment on dietary fibre: A collaborative study. *JAOAC* (submitted).
- Wahlqvist, M. L., Wilmshurst, E. G., Murton, C. R. and Richardson, E. N. (1978). The effect of chain length on glucose absorption and the related metabolic response. *Am. J. Clin. Nutr.*, **31**, 1998-2001.
- Wells, G. B. (1976). The role of dry milled cereal products in fabricated foods. *Cereal Foods World*, **21**, 14-18.
- Wolf, J. C., Thompson, D. R. and Reineccius, G. A. (1977). Kinetics of available lysine loss during thermal processing of a soy protein isolate; reversion of no-loss to rapid-loss phase. *J. Food Proc. and Preserv.*, **1** (4) 271-9.
- Wolf, J. C., Thompson, D. R. and Reineccius, G. A. (1978). Comparisons between model predictions and measured values for available lysine losses in a model food system. *J. Food Sci.*, **43** (5), 1486-90.
- Wolf, J. C., Thompson, D. R., Ahn, P. C. and Hegarty, V. J. (1979). Kinetics of available lysine losses in a soy protein isolate: confirmation of the 'transition phase' by protein efficiency ratio tests. *J. Food Sci.*, **44** (1), 294-5.

3 PURPOSE AND DESIGN OF THE PRESENT STUDY

The purpose of the present study was to evaluate the effect of HTST twin-screw extrusion cooking on some nutritional properties of wheat products.

The following parameters were studied:

protein nutritional value evaluated by amino-acid analysis and nitrogen-balance studies in rats

availability of starch for digestion and absorption as well as for fermentation in human dental plaque

dietary fibre content, composition and fermentability in the rat intestine.

Comparisons were made with the corresponding raw materials. In addition, some studies were performed on relevant alternative processes such as boiling, drum-drying and baking. The equipment used for drum-drying was a pilot-plant single drum (Goudsche Machinefabriek B.V., Gouda, Holland). Boiling was performed in a water bath for 20 minutes, and baking, in a traditional oven.

A brief summary of the materials and nutritional parameters studied is given in Table 3.

Table 3 MATERIALS AND PARAMETERS STUDIED

	Protein Nutritional Value			Starch Availability			Dietary Fibre	
	Amino-acids	Available lysine	Nitrogen balance (rats)	Digestion (in vitro)	Digestion & Absorption (rats) (man)	Fermentation in dental plaque (man)	Content and/or composition	Fibre Fermentation in the colon (rats)
Wheat Starch								
extruded				x				
boiled				x				
raw				x				
Wheat Flours								
extruded	x		x	x		x	x	x
boiled	-		-	x		x	-	-
drum-dried	-		-	x		x	x	-
raw	x		x	x	-	x	x	x
Whole-Grain Flours								
extruded	x		x	x			x	x
boiled	-		-	x			-	-
raw	x		x	x	-		x	x
Biscuits								
extruded	x	x	x					
raw mixture	x	x	x					
Wheat Flour								
Breads								
extruded					x		x	
baked					x		x	
Whole-Grain Wheat Breads								
extruded					x		x	
baked					x		x	

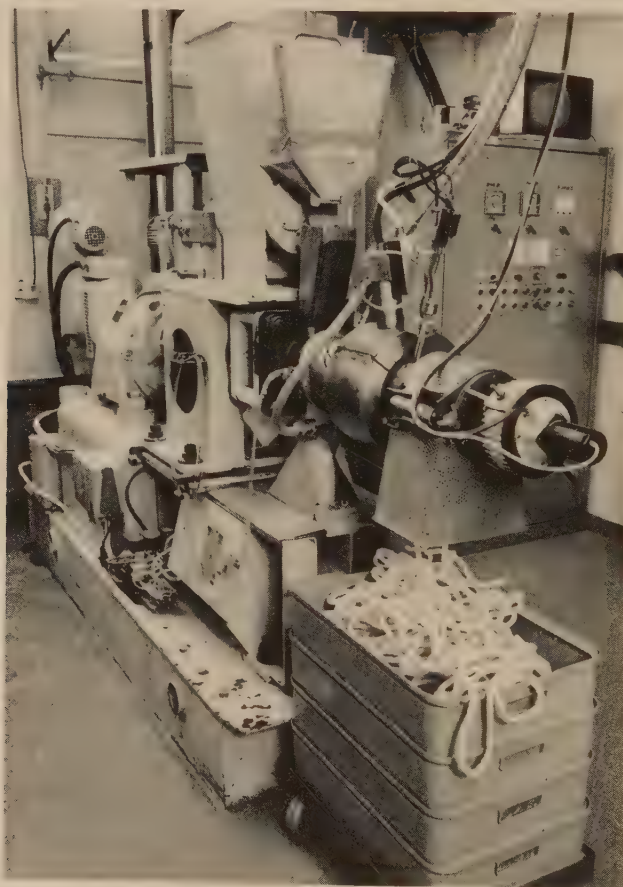
3.1 EXTRUSION EQUIPMENT AND PROCESS CONDITIONS

A Creusot-Loire BC 45 twin-screw extruder was used throughout the study (Fig 3).

The extruder was equipped with co-rotating intermeshing screws. The screw profiles consisted of five segments; four compression screw elements and one reverse flighted element.

The range of extrusion conditions used are summarized in Table 4.

Fig. 3



A Creusot-Loire BC 45 twin-screw extruder (reproduced with permission from SIK - The Swedish Food Institute)

Table 4

EXTRUSION CONDITIONS (Creusot-Loire BC 45 twin-screw extruder)

	Feed rate (kg/h)	Moisture content (%)	Mass ^a temp. (°C)	Screw speed (rpm)	Screw ^b composition	Die ^c geometry (mm)	Residence ^d time (s)	Pressure ^a (bar)
<u>Pre-cooked</u>								
<u>Wheat Products</u>								
wheat starch	12	15						
wheat flour	or	or	156-180	100-200	FCCCCRD	Ø 5		
whole-grain flour	21	20				ℓ 27		
<u>Biscuits</u>								
(wheat flour, corn starch, soy protein isolate, sodium caseinate, sucrose and NaCl)	40	13 or 18	170-210	80	FCCCRCD	h 1.5 w 22.3 ℓ 47	42-44	75-200
<u>Crispbread Type Products</u>								
wheat flour	26	11	159			h 2.0		130
whole-grain wheat flour	or	or	or	150	FCCCCRD	w 20		or
	27	13	165			ℓ 27		76

a Mass temperature and pressure were measured in the compression chamber just before the die

b Screw composition; F (feed), D (die), C (compression element) and R (reverse flighted element)

c Die geometry; Ø (diameter), ℓ (length), h (height) and w (width)

d Residence time was determined by adding erythrosin to the raw material

4.1 PROTEIN NUTRITIONAL VALUE

One of the main mechanisms responsible for protein deterioration during heat treatment of food is the Maillard reaction (1). This reaction, between reducing sugars and protein, leads to a decrease in the availability of lysine in particular as well as in protein digestibility.

Mainly cereal-based products are processed in extrusion cookers. As lysine is limiting in cereals, and appeared to be the most reactive amino-acid in the products studied (I, II), this investigation was focused on lysine retention.

From the nutritional as well as from the analytical point of view, the Maillard reaction can be divided into "early" and "advanced" reactions. The initial step involves the condensation of a carbonyl group of a reducing sugar and a free amino group (2). The high reactivity of protein-bound lysine is due to its free ϵ -amino group. The "early" reactions include the formation of the Amadori compound, that is the 1-amino-1-deoxy-2-ketosyl derivative. In some food systems the Maillard reaction stops at this point (3). The ϵ -N-deoxy ketosyl derivative of lysine cannot be used as a source of lysine by the rat (4 - 6), and is also considered unavailable in man. This unavailable lysine derivative constitutes the major form of blocked lysine in processed food (7). After early Maillard reactions, there is little change in the colour of the product. In the "advanced stages", however, coloured products are formed. In addition, there is a general decrease in amino-acid availability, caused by the formation of enzyme resistant cross-links (3). At this stage the mechanism of lysine damage is destruction rather than blocking of the ϵ -amino group, due to degradation of the deoxy ketosyl derivative, or to the reaction of lysine with active intermediate compounds (Strecker degradation) (7).

Determination of the total lysine content after acid hydrolysis, is a poor measure of availability after early Maillard reactions. During acid hydrolysis blocked lysine is split, thus regenerating free lysine (8). In order to circumvent this problem, several chemical methods have been suggested for the determination of available lysine in processed food (9 - 12). Some of these, e.g. the FDNB (1-fluoro-(2,4)-dinitro benzene) method (9), involve the use of a reagent that reacts with the free ϵ -amino group of lysine. The coloured lysine derivative formed can be used as a measure of availability. The FDNB-method is considered one of the most reliable for the determination of available lysine after Maillard reactions (7, 13). However, chemical determinations of available

lysine, or more correctly "reactive" lysine, fail to predict losses in in vivo availability in cases where the protein digestibility is affected. Lysine with a free "reactive" ϵ -amino group can still be physiologically unavailable if adjacent linkages are not split in the digestive tract.

Another important feature of the Maillard reaction is the formation of flavour and colour. The product colour is comparatively easy to measure, and may provide information about the extent of lysine damage. However, colour is not a relevant measure of protein quality in the early stages of the Maillard reaction, where mainly uncoloured products are formed.

During high-temperature short-time baking of pizza crust (14), and balady bread (15), there was a similar decrease in total and available lysine, indicating that the mechanism of lysine damage was destruction. On the other hand, when comparing oven baking, microwave baking and steaming, oven baking significantly reduced the availability of lysine (16). However, only minor differences were observed in the total lysine content. Thus, depending on the food process and/or process conditions, both the nature and the extent of lysine damage may differ.

4.1.1 Lysine retention

The first paper (1) reports effects on lysine retention during extrusion cooking of a protein-enriched biscuit. The ingredient mixture contained wheat flour, corn starch, soy-protein isolate, sodium caseinate, sucrose and sodium chloride. The products were processed under different conditions regarding temperature and moisture content of the raw material. Amino-acid composition, including total lysine, was analyzed by ion-exchange chromatography after acid hydrolysis. "Reactive" lysine was analyzed by the FDNB method as described by Noguchi et al. (17). In addition, the content of biologically available lysine was evaluated from nitrogen-balance studies in rats (18). Lysine was made limiting in test diets by mixing the products with wheat gluten. In order to obtain a quantitative measure of available lysine, balance experiments were performed on standard diets with known amounts of lysine. The standard diets contained free amino-acids and wheat gluten in the same proportions as in the test diets. From the standard graphs obtained, $BV = f(\text{"added" lysine})$ and $NPU = f(\text{"added" lysine})$, it was possible to calculate the content of available lysine in the different products. Calculations were performed both from the biological value (BV) and the net protein utilization (NPU) of the test diets, the NPU also taking into account effects on true protein digestibility (TD).

Under the mildest conditions used, the lysine loss was close to 11 % regardless of analytical method. The similar decreases in total and available lysine indicate that the mechanism of lysine damage was destruction rather than blocking of the ϵ -amino group. Under more severe conditions, however, there was a discrepancy between the lysine loss indicated by the chemical methods, 37 %, and the NPU values, 50 %. The deviation was partly due to a reduction in TD.

FDNB-reactive lysine and available lysine content calculated from the BV, both fail to measure true availability if the overall protein digestibility (TD) is affected. These methods could therefore be expected to give similar results. This was the case in the raw material and in the product processed with a high moisture content. In the other products, calculation of lysine content from the BV gave consistently 10 to 20 % lower values. This discrepancy with some of the extruded products, could nevertheless be due to a reduced availability to proteolytic enzymes in vivo. Small unavailable peptides may be absorbed intact by the rat and excreted in the urine (19, 20). Absorption and subsequent excretion of unavailable peptides containing reactive lysine could explain the lower values obtained when calculating the lysine content from the BV. It has also been suggested that Maillard compounds as such may impair the utilization of protein in vivo (21). Recently an in vitro method was published, using physiological enzymes, instead of HCl, to hydrolyze food proteins (22). Levels of available lysine analyzed with this method were in good agreement with results obtained in the animal assay (23). This indicates that, in addition to destruction, a reduced availability to proteolytic enzymes could be a mechanism behind the loss of available lysine in the products studied (I).

Under the mildest extrusion conditions used, the lysine loss is close to that reported during the baking of bread (24), whereas the loss under severe conditions is comparable to that during baking of a similar biscuit (25). During industrial production of extruded bread products, additional heat treatment is often performed to improve the flavour and colour of the product. No such heat treatment, that could decrease the protein nutritional value further, was performed in this study. However, other heat-sensitive nutrients, such as vitamins, have been reported to remain almost unaffected during after-drying of extruded crispbread (26).

It has been suggested that the rate of the Maillard reaction during extrusion cooking of a biscuit with a similar recipe to that used in the first paper (I), is limited by the formation of reducing sugars through sucrose hydrolysis (17).

The materials studied in the second paper (II) were wheat flour and whole-grain wheat flour, without additional carbohydrates present. Amino-acid composition was analyzed by gas-liquid chromatography, after acid hydrolysis and a subsequent derivatization to N-trifluoro-acetyl-n-butyl derivatives (27). The protein nutritional value was evaluated through nitrogen-balance studies in rats (18). In addition, colour measurements were performed.

The loss of total lysine ranged from 0 to 37 %, depending on process conditions. The loss of lysine in whole-grain wheat flour was accompanied by a decrease in TD, whereas no such effect was seen in the wheat flour products. This observation is difficult to interpret, as the lysine damage in some of the wheat flour products was much more prominent than in the whole-grain products. However, it has been suggested that the intense mixing during extrusion cooking may reduce the protein digestibility due to interactions between protein and undigestible polysaccharides present in the cell walls (28).

A correlation was found between the lysine content and the degree of browning in the processed wheat flour products. However, the colour could not be used to predict the lysine loss relative to the raw material. This provides evidence against the reliability of reflectance measurements for the evaluation of protein quality in extruded products. Possibly other factors than Maillard products affect the reflectance, e.g. starch gelatinization.

The rate of the Maillard reaction is enhanced by high temperature and intermediate water activities (29, 30). It is not possible to measure the water activity inside an extruder. However, at elevated temperatures and pressures, which occur during HTST extrusion cooking, a high water activity is probably achieved even at comparatively low water content (28).

In general, there was a decrease in lysine content with increasing mass temperature (I and II). An increase in moisture content of feed, at a constant mass temperature, had a beneficial effect on lysine retention (I). This might be explained by the law of mass action, as water is produced in the first reversible phase, initiating the Maillard reaction (17).

Very few studies have been performed to evaluate the impact of feed rate on nutrient retention. In this study (II), it was found that an increase in feed rate significantly improved the protein nutritional value of the product. This was probably due to a reduced residence time (31).

An increase in screw speed, from 150 to 200 rpm, increased the lysine loss (II) which is in agreement with results obtained by Tsao (32) during extrusion cooking of rice meal in a single-screw extruder. The increased loss at a higher screw speed was attributed to an increased degradation of starch. Dextrinization of starch has also been reported during twin-screw extrusion cooking (33, 34). Formation of reducing saccharides, through hydrolysis of starch, may be a mechanism behind the prominent lysine loss in wheat flours, processed under severe extrusion conditions (II).

4.2 STARCH AVAILABILITY

Cooking and gelatinization of starch increase the susceptibility to enzymic degradation in vitro (35, 36), as well as the availability for digestion and absorption in the small intestine (37, 38). Processing can also affect the availability of carbohydrates in a food item, by mechanically disrupting the organized structure, thus increasing the surface area/starch ratio (39, 40).

During the last few years, much attention has been focused on the availability of starch in various food items (41, 42). A reduced rate of carbohydrate absorption is considered beneficial in the control of diabetes. It has also been suggested that any dietary change that leads to a more rapid absorption of carbohydrates, promotes the development of obesity (43). Due to the presence of α -amylase in saliva, an increased availability of starch in processed foods could also favour development of dental caries. In the mouth, starch is hydrolyzed mainly in the saliva and, to a minor extent within the plaque (44 - 47). The low molecular weight dextrins formed diffuse into the plaque and are metabolized to organic acids by the micro-organisms present, with a resulting drop in plaque pH.

The term gelatinization is normally used to describe the swelling of starch granules during the heating of starch-water systems in the presence of excess water. This water-mediated melting of wheat starch, does not take place at water contents below 33 % under normal conditions (atmospheric pressure, low shear rates) (48). However, during extrusion conditions, starch granules lose their crystallinity at a considerably lower water content (49 - 51).

Burt and Russel (52) used differential scanning calorimetry (DSC) and light microscopy to study the gelatinization of wheat starch at low water content, 1.5 - 50 %. As the water content was reduced, the temperature needed to lose the birefringence of starch granules, became progressively higher. It was suggested that melting of starch in the absence of water, would require tempe-

ratures above 232° C. Thus, starch is gelatinized through different mechanisms, depending on the amount of water present.

The mechanism of gelatinization, or rather loss of starch crystallinity, during extrusion cooking is not fully understood. In addition to the heat treatment, the intense mechanical treatment in an extruder probably affects the starch granules (51, 53).

Dextrinization of starch has been reported in several papers (53 - 57, 33, 34). This also occurs during other processes, such as baking. However, at low moisture content, the hydration of liberated linkages may not be complete, and the presence of highly reactive anhydro-dextrins was recently observed in extruded wheat products (58).

To what extent different processes affect starch availability is not well documented. The present investigation was performed to study the effect of HTST extrusion cooking on starch availability in wheat products. Comparisons were made with other processes commonly used for gelatinization, such as boiling (III), drum-drying (IV) and baking (V).

4.2.1 Digestion with salivary amylase in vitro

Starch content was analyzed with a glucose oxidase reagent (59) after incubation with amyloglucosidase. The availability of starch to enzymic digestion in vitro was evaluated by incubation with human salivary amylase. The products formed were quantified at regular time intervals during 2 h with a 3,5-dinitro-salicylic acid reagent (DNS) (60). The degree of hydrolysis was expressed as maltose equivalents.

Starch in raw wheat flour and raw whole-grain wheat flour was hydrolyzed very slowly in vitro. The degree of hydrolysis after 2 h incubation was less than 5 % (III). Extruded wheat starch, or starch in extruded wheat flour and whole-grain wheat flour products, was hydrolyzed very rapidly. The degree of hydrolysis in these materials reached a plateau corresponding to about 80 %. The extrusion conditions did not affect the in vitro availability. Flours that were gelatinized by boiling in water were less susceptible to amylase, the degree of hydrolysis levelling off at 40 - 50 % (III, IV). Boiling of pure wheat starch, on the other hand, rendered the starch as available as starch present in extruded products (III).

The difference in availability between boiled pure starch and starch in boiled flours was possibly due to the more complex structure of flour. The importance of the protein structure, for the enzymic availability of starch in boiled wheat flour, was recently shown (38). By introducing a pre-incubation with pepsin prior to incubation with α -amylase, a significant increase in starch availability was obtained. In contrast, no effect of pepsin was seen in wheat products processed by popping, flaking or steaming. The importance of the protein structure has also been demonstrated with other cereals. According to Mc Neill et al. (61), the solubility of the protein matrix encapsulating the starch granules in sorghum, was even more important for the enzymic availability of starch than the degree of gelatinization.

In contrast to extruded materials, the starch in drum-dried wheat flours was less available to salivary amylase than starch in boiled flour (IV). Drum-drying resulted in a slower initial rate of amylolysis. After prolonged incubation, however, the degree of hydrolysis reached the level seen in boiled flour. The conditions during drum-drying affected the availability; more severe conditions resulting in a higher rate of amylolysis. Boiling or homogenization of suspensions of drum-dried wheat flour, made the starch as available as in boiled flour.

The differences in enzymic availability of starch in extruded, drum-dried and boiled wheat products, cannot be explained by differences in the degree of gelatinization. All extruded wheat products were completely gelatinized, as judged by the absence of intact starch granules in scanning electron photomicrographs (62). Further, no endothermic transition, corresponding to starch gelatinization, could be detected by differential scanning calorimetry (DSC) in boiled or drum-dried wheat flours (IV). However, with drum-dried and boiled wheat flour, an endothermic peak appeared, corresponding to the dissociation of amylose-lipid complexes. The transition enthalpy (ΔH) was particularly pronounced in the case of drum-drying. In addition, with drum-dried flours the transition occurred within a very narrow temperature interval, indicating that the complexes formed during drying were more stable and more highly ordered than those formed during boiling. As judged from the decrease in ΔH , reheating or homogenization of suspensions of drum-dried flour reduced the amount of amylose-lipid complexes and/or modified them into a less ordered structure. Amylose-lipid complexes dissociate at high temperature, but reform on cooling (63). This study (IV) showed that once disrupted by heat or shear, the characteristic complexes formed during drum-drying do not reform.

Amylose-lipid complexes are much less available for enzymic digestion than free amylose (64). During gelatinization, amylose leaks from the starch granules (63). Encapsulation of starch with complexed amylose during drying, could be a mechanism behind the lower availability of starch in drum-dried wheat flour. Formation of amylose-lipid complexes has also been reported during extrusion cooking (50, 65). In the present study, some results indicate the presence of complexed amylose in extruded whole-grain flour (see 4.3.1). However, it is probable that the complexes, formed during extrusion, become evenly distributed in the product due to the intense mixing, and would thus be expected to have less influence on the starch availability. Recently, Holm et al. (66) also observed highly ordered amylose-lipid complexes in drum-dried wheat flour by DSC. In contrast, no complexed amylose was detected after extrusion cooking of the same material. The importance of amylose-lipid complexes for the availability of starch in processed products and the conditions necessary for their formation, are issues that need further investigation.

When comparing extrusion cooking and drum-drying for gelatinization of wheat starch, differences have been reported regarding effects on the starch molecular weight (67). Extrusion cooking under HTST conditions, led to degradation of amylose and amylopectin, by random chain splitting, whereas drum-drying only had minor effects in this respect. Degradation of starch would be expected to increase the availability to α -amylase, particularly as amylopectin appears to be affected to a greater extent than amylose during extrusion (34, 68). Further, at a water absorption index suitable for preparation of gruels, the solubility index of corn-grits was higher after extrusion cooking than after drum-drying (69). It is likely that the degree of solubilization affects the enzymic availability.

The rate and extent of in vitro starch digestion was significantly higher after extrusion cooking than after boiling or drum-drying in this study (III, IV). The differences in availability to α -amylase may result from differences in starch solubility and/or the solubility of the protein matrix.

4.2.2 Fermentation in human dental plaque in situ

Due to the high susceptibility of starch in extruded products to salivary amylase (III), these products would be expected to provide the dental plaque micro-organisms with readily fermentable dextrins. To study this aspect, subjects rinsed their mouths for 30 s with 10 ml of various test suspensions. Samples of dental plaque were taken for pH determination immediately before and on different occasions after rinsing (70). The following test suspensions

were used, containing an equivalent amount of carbohydrate (5 % w/v): glucose, boiled soluble starch, raw wheat flour, extruded wheat flour (mild and severe conditions) and drum-dried wheat flour (mild conditions) (III, IV).

Glucose produced the most prominent drop in plaque pH, followed by boiled soluble starch (III). The pH drop after rinsing with suspensions of extruded products was significantly more pronounced than with a boiled wheat flour control (III). No difference in pH drop was observed due to extrusion conditions. In contrast, the pH drop after rinsing with suspensions of drum-dried flour, was similar to that obtained with a boiled control (IV). Raw wheat flour only had minor effects on plaque pH. Thus, although the differences in availability to salivary amylase were much more pronounced in vitro than in situ, a similar tendency was obtained.

A pH drop below 6.5 can be considered critical for caries development with the method used for collection of plaque (71). Based on this figure, all processed products produced critical pH-values in the plaque. However, the pH recovery was more rapid after rinsing with suspensions of drum-dried or boiled flour, than with suspensions of extruded wheat flour products.

The results indicate (III) that extrusion cooking makes the starch easily fermentable in plaque, approximately to the same extent as boiled soluble starch. Extruded products must therefore be considered as potentially cariogenic, especially if they are consumed frequently, or retained for long periods in the mouth. It has been shown that the contact time between starch and saliva is a crucial factor for the drop in plaque pH (44). Consequently, a reduced stickiness of extruded products must be considered beneficial in connection with dental health.

4.2.3 Plasma glucose and insulin responses in rats

To study the importance of the differences in in vitro starch availability (see 4.2.1), for the rate of uptake in vivo, rats were given suspensions of extruded, boiled and drum-dried wheat products by gastric intubation (III, IV). An equivalent amount of starch (100 mg) was given after an overnight fast, and blood samples were taken at regular time intervals from the retro-orbital sinus (72) for the determination of plasma glucose (73) and immuno-reactive insulin (74).

The plasma glucose and insulin responses to wheat flour and whole-grain wheat flour, extruded under mild conditions, did not differ from those obtained with

the corresponding boiled materials (III). In contrast, flours extruded under more severe conditions (higher temperature and screw speed) produced a significantly higher early glucose response. There was also a tendency towards a corresponding increase in insulin response. Thus, the conditions during extrusion cooking can influence the glycemic response to starch. The increase in response under severe extrusion conditions must be considered small. However, it could have been more pronounced in animals with impaired glucose tolerance.

Drum-drying of wheat flour resulted in a significantly lower plasma glucose response than boiling (IV). In agreement with the plasma glucose pattern, the early insulin increment was lower with drum-dried flours. Boiling of drum-dried wheat flour increased the peak glucose response above the level seen after boiling alone.

The inconsistency between in vitro and in vivo results, when comparing boiled and extruded products, indicates that the efficiency of amylolysis in vivo is high enough to eliminate the differences observed in vitro, or that other factors, apart from the availability to α -amylase, were of major importance for the rate of digestion and absorption in vivo (III). The agreement might have been better if, for instance, proteolytic enzymes were included in the in vitro system (38).

In contrast, when studying starch availability in drum-dried and boiled wheat flours, a good correlation was obtained in vitro and in vivo (IV). Thus, the lower rate of digestion and absorption of starch in drum-dried flour, can be explained by assuming that starch digestion as such was rate-limiting. A possible mechanism could be the encapsulation of starch with highly crystalline amylose-lipid complexes during drying (see 4.2.1).

4.2.4 Post-prandial glucose and hormonal responses in non-insulin-dependent diabetics

In the animal assay previously discussed (see 4.2.3), the extrusion conditions influenced the glycemic response to wheat products in healthy rats. Study V was performed to evaluate the post-prandial glucose and hormonal responses in non-insulin-dependent diabetics given extruded or baked bread as the main source of carbohydrate in a breakfast meal.

Baking and extrusion cooking were performed with wheat flour and whole-grain wheat flour, respectively. The extruded products had the character of crispbread. Nine patients were included in the study. None had any antidiabetic

treatment. The patients were given the four types of bread, corresponding to an equivalent amount of starch, in a realistic composite breakfast. In addition to bread, the meals contained milk, margarine, soft cheese, tea or coffee and water. The study started in the morning after an overnight fast, and the time taken to complete the meals was on average 19 minutes. Samples of venous blood were drawn at 10 to 30-minute intervals until 3 hours after the meal. The following parameters were analyzed: blood glucose (75), and in plasma; immuno-reactive insulin (74), connecting peptide (76), gastric inhibitory polypeptide (77) and glucagon (78).

The conditions used during extrusion cooking of the crispbreads (V) must be considered very mild. Nevertheless, intake of a breakfast containing extruded whole-grain crispbread resulted in somewhat higher post-prandial responses than a breakfast with the corresponding baked bread. The areas under the curves for blood glucose and plasma insulin were significantly higher with the extruded whole-grain product: No significant differences were obtained when comparing extruded and baked bread made from white wheat flour. However, the breakfast containing white crispbread was difficult to complete within the allotted time. The slower ingestion of the white crispbread breakfast may have concealed a higher availability of starch in this product.

The post-prandial responses to white wheat bread and whole-grain wheat bread were not significantly different, although a tendency to a lower response after whole-grain bread was evident. Thus, the amount of naturally occurring dietary fibre in wheat has little effect on the glycemic response after a single meal. This is consistent with the results in the rat assay (III), where the plasma glucose and insulin responses to suspensions of wheat flour and whole-grain wheat flour were similar.

Considerable amounts of starch granules remain intact during baking, particularly in the crust (79). The higher post-prandial responses with extruded rather than with baked whole-grain bread may be due to differences in the degree of gelatinization. Although the difference in the rate of starch absorption was small, baked bread is to be preferred to extruded products for patients with type II diabetes.

4.3 DIETARY FIBRE

Dietary fibre includes non-starch polysaccharides and lignin, which are resistant to the enzymes present in the digestive tract. A number of physiological effects have been attributed to dietary fibre, such as increased faecal volume,

reduced transit time, improved glucose tolerance and lowered plasma lipids. (80).

Only few reports exist on the effects of processing on dietary fibre content or on the physiological properties listed above. Another aspect on starch availability, than those discussed previously (see 4.2), is in relation to dietary fibre analysis. All analytical procedures for the determination of dietary fibre are dependent on an efficient degradation of starch. Even if only a small fraction of the starch in cereals was rendered enzyme-resistant during processing, this would lead to a considerable increase in apparent dietary fibre content. In addition, Maillard products formed during heat treatment (see 4.1), will be partly recovered as "lignin" (81).

The high bulking capacity of wheat fibre, is mainly due to its high resistance to bacterial fermentation in the colon (82). Cooking, as well as reduction of particle size, has been shown to diminish the beneficial effect of wheat bran in this respect (83 - 86).

Study VI, was performed to evaluate the effect of HTST extrusion cooking on dietary fibre in wheat flour (80 % extraction rate) and whole-grain wheat flour. Comparisons were made with drum-drying (IV).

4.3.1 Dietary fibre content

Dietary fibre (DF) content was analyzed gravimetrically after enzymic solubilization of protein and starch (87). This method involves pre-incubation with a thermostable bacterial α -amylase during gelatinization, prior to incubation with physiological enzymes (pepsin, pancreatin). Insoluble polysaccharides are recovered by filtration and soluble ones after precipitation with ethanol, and subsequent filtration. The fibre residues obtained are corrected for remaining protein ($6.25 \cdot N$) and ash. Thus, Maillard compounds are not determined as DF with this method.

An increase in DF content of about 1 % (dry basis), was observed in all extruded whole-grain wheat products (VI). Extrusion cooking, as well as drum-drying of white wheat flour, produced a similar increase in DF content, but only at severe process conditions (IV, VI).

The slight increase in total DF content in this study is comparable to that recently reported during extrusion cooking of wheat flour (58), during baking of bread (88) and heat treatment of potatoes (89). However, if the thermostable

α -amylase (Termamyl) was excluded from the gelatinization step in the DF assay, the increase in apparent DF content during extrusion cooking was considerable, up to 6 % (VI). This increase in DF content, analyzed without Termamyl, could in part be due to the presence of amylose-lipid complexes, as these complexes are hydrolyzed by Termamyl, but only slowly attacked by amylases at low temperature (64). In addition, extrusion cooking of the whole-grain wheat products reduced the amount of fat that could be extracted with chloroform/methanol (90). According to Holm et al. (64), amylose-lipid complexes are completely digested in vivo, and should therefore not be included as DF. This emphasizes the importance of an efficient starch digestion when analyzing DF in processed products.

Extrusion cooking of wheat flour caused a significant change in the ratio of insoluble to soluble DF. In raw flour 40 % of the total DF was soluble, whereas 50 - 75 % was soluble after extrusion cooking. The solubilization appeared to be dependent on process conditions. In the wheat product processed at a higher feed rate, the solubilization was less pronounced. The main fibre constituents in wheat, arabinose, xylose and glucose, were solubilized to approximately the same extent (VI). An increase in soluble DF was also observed in extruded whole-grain products. However, the change in proportions of insoluble and soluble DF was not statistically significant. The increase in soluble dietary fibre during extrusion could be interpreted as a result of the breaking of chemical bonds within dietary fibre polysaccharides.

4.3.2 Fermentation in the rat intestine

The susceptibility of dietary fibre (DF) to fermentation by colonic bacteria was studied in rats given raw or extruded wheat products (VI). Fibre-balance experiments were performed as previously described (91). The DF components in feed and in faeces were analyzed by gas-liquid chromatography as alditol acetate derivatives (92).

The balance experiments showed that the DF in raw wheat flour was fermented to a high degree. However, extrusion cooking, even if performed under mild conditions, increased the fermentability further (VI). The faecal recovery of the main components, arabinose, xylose and glucose, averaged 22 % in raw wheat flour, versus 12 % after extrusion cooking. In contrast, with whole-grain wheat flour, the recovery of DF components in faeces was similar in raw and extruded material, about 40 %.

In general, soluble DF is more easily fermented than insoluble fibre (93). The significant redistribution between soluble and insoluble DF in extruded wheat flour (see 4.3.1), might be a factor increasing the fermentability. Since white flour cannot be considered an important source of DF, a diminished bulking capacity which may follow, should not be of nutritional significance. The fact that the fermentability of DF in whole-grain flour was not altered, indicates that extrusion cooking primarily affects a smaller fibre fraction connected with the endosperm. This may not be revealed in the case of whole-grain wheat flour, due to its higher DF content.

Only minute amounts of starch remained in the faeces of rats given raw or extruded wheat products, indicating that the overall digestibility of starch was close to 100 % (VI). This assay does not distinguish between enzymic degradation and bacterial fermentation in the colon. However, very small amounts of starch were detected in the ileostomy evacuate of patients given extruded wheat products, indicating almost complete digestion (94). On the other hand, recent studies have shown that up to 20 % of the starch in a meal may be passed undigested to the colon in human subjects (95, 96). The rate and extent of digestion and absorption of starch from various sources, and the effect of processing, are important fields for further studies.

LITERATURE CITED (4.1 - 4.3)

1. Cheftel, J.-C. (1979) Proteins and amino acids. In: Nutritional and Safety Aspects of Food Processing (S.R. Tannenbaum, ed.) Marcel Dekker Inc., New York and Basel, pp 153 - 215.
2. Hodge, J.E. (1953) Agric. Food Chem. 15, 928 - 943.
3. Mauron, J. (1981) The Maillard reaction in food - A critical review from the nutritional standpoint. In: Maillard Reactions in Foods. Progress in Food and Nutrition Science. Vol 5. (C. Eriksson, ed.) Pergamon Press Ltd., pp 5 - 35.
4. Finot, P.A. and Mauron, J. (1969) Helv. Chim. Acta 52, 1488 - 1495.
5. Mauron, J. (1970) Int. J. Vit. Nutr. Res. 40, 209 - 227.
6. Finot, P.A., Magnenat, E., Motter, F. and Bujard, E. (1978) Ann. Nutr. Alim. 32, 325 - 338.
7. Hurrell, R.F. and Carpenter, K.J. (1981) The estimation of available lysine in foodstuffs after Maillard reactions. In: Maillard Reactions in Foods. Progress in Food and Nutrition Science. Vol 5. (C. Eriksson, ed.) Pergamon Press Ltd., pp 159 - 176.
8. Finot, P.A. (1973) Non-enzymatic browning. In: Proteins in Human Nutrition. (J.W.G. Porter and B.A. Rolls, eds.) Academic Press, London, pp 501 - 514.
9. Carpenter, K.J. (1960) Biochem. J. 77, 604 - 610.
10. Eklund, A. (1976) Analyt. Biochem. 70, 434 - 439.
11. Hurrell, R.F., Lerman, P. and Carpenter, K.J. (1979) J. Food Sci. 44, 1221 - 1227.
12. Maga, J.A. (1981) J. Food Sci. 46, 132 - 134.
13. Erbersdoubler, H.F., Anderson, T.R. (1983). Determination of available lysine by various procedures in Maillard-type products. In: The Maillard Reaction in Foods and Nutrition (G.R. Waller and M.S. Feather, eds.) ACS

Symposium Series 215, American Chemical Society, Washington, pp 419 - 427.

14. Tsen, C.C., Bates, L.S., Wall, L.L. and Gehrke, C.W. (1982) J. Food Sci. 47, 674 - 675.
15. Tsen, C.C., Reddy, P.R.K., El-Samahy, S.K. and Gehrke, C.W. (1983) Effect of the Maillard browning reaction on the nutritional value of breads and pizza crusts. In: The Maillard Reaction in Foods and Nutrition (G.R. Waller and M.S. Feather, eds.) ACS Symposium Series 215, American Chemical Society, Washington, pp 379 - 394.
16. Tsen, C.C., Reddy, P.R.K. and Gehrke, C.W. (1977) J. Food Sci. 42, 402 - 406.
17. Noguchi, A., Mosso, K., Aymard, C., Jeunink, J. and Cheftel, J.-C. (1982) Lebensm.-Wiss. Technol. 15, 105 - 110.
18. Eggum, B.O. (1973) A study of certain factors influencing protein utilization in rats and pigs. "406 Beretning fra Forøgslaboriet". Statens Husdyrbrugsudvalg, Copenhagen, Denmark.
19. Valle-Riestra, J. and Barnes, R.H. (1970) J. Nutr. 100, 873 - 882.
20. Pronczuk, A., Pawlowska, D. and Bartnik, J. (1973) Nutr. Met. 15, 171 - 180.
21. Adrian, J. (1974) World Rev. Nutr. Diet. 19, 71 - 122.
22. Matoba, T., Doi, E., Yonezawa, D., Üste, R. and Nair, B.M. (1982) Agric. Biol. Chem. 46, 465 - 472.
23. Björck, I., Matoba, T. and Nair, B.M. (1984) In-vitro enzymatic determination of the nutritional value of proteins and amount of available lysine in some cereal food products processed by extrusion cooking. Agric. Biol. Chem. (submitted)
24. Rosenburg, H.R. and Rohdenburg, E.L. (1951) J. Nutr. 45, 593 - 598.
25. Carpenter, K.J. and March, B.E. (1961) J. Nutr. 15, 403 - 410.

26. Millauer, C., Wiedmann, W.M. and Killeit, U. (1983) Influence of different extrusion parameters on the vitamin stability. Abstract. COST 91. Concluding Seminar "Thermal Processing and Quality of Foods", Athens 14 - 18 November, Greece.
27. Nair, B.M. (1977) J. Agric. Food Chem. 25, 614 - 620.
28. Linko, P., Colonna, P. and Mercier, C. (1981) High-temperature, short-time extrusion cooking. In: Advances in Cereal Science and Technology (Y. Pomeranz, ed.) American Association of Cereal Chemists Inc., St. Paul, Minnesota, pp 145 - 235.
29. Lea, C.H. and Hannan, R.S. (1949) Biochem. Biophys. Acta 3, 313 - 325.
30. Eichner, K. (1975) The influence of water content on non-enzymic browning reactions in dehydrated foods and model systems and the inhibition of fat oxidation by browning intermediates. In: Proceedings of an International Symposium on Water Relations in Foods., Glasgow 1974. (R.B. Duckworth, ed.) Academic Press, London, pp 417 - 434.
31. Olkku, J., Antila, J. and Heikkinen, J. (1980) Residence time distribution in a twin screw extruder. In: Food Process Engineering. Vol I. Food Processing Systems (P. Linko, Y. Mälkki, J. Olkku and J. Larinkari, eds.) Applied Science Publishers Ltd., London, pp 791 - 794.
32. Tsao, T.-F. (1976) Available lysine retention during extrusion processing. PhD Dissertation, Colorado State University, Colorado, USA.
33. Kim, J.-C. (1983) Effect of some extrusion parameters on the solubility and viscograms of extruded wheat flour. Abstract. COST 91. Concluding Seminar "Thermal Processing and Quality of Foods", Athens 14 - 18 November, Greece.
34. Meuser, F. and van Lengerich, B. (1983) System analytical model for the extrusion of starches. Abstract. COST 91. Concluding Seminar "Thermal Processing and Quality of Foods", Athens 14 - 18 November, Greece.
35. Snow, P. and O'Dea, K. (1981) Am. J. Clin. Nutr. 34, 2721 - 2727.
36. Delort-Laval, J. and Mercier, C. (1976) Ann. Zootech. 25, 3 - 12.

37. Collings, P., Williams, C. and Mc Donald, J. (1981) Br. Med. J. 282, 1032.
38. Holm, J. Asp, N.-G. and Lundquist, I. (1984) Digestibility of starch in vitro and in vivo after flaking, steaming and popping of wheat flour (to be published).
39. O'Dea, K., Nestel, P.J. and Antonoff, L. (1980) Am. J. Clin. Nutr. 33, 760 - 765.
40. Collier, G. and O'Dea, K. (1982) Am. J. Clin. Nutr. 36, 10 - 14.
41. Crapo, P.A., Reaven, G. and Olefsky, J. (1977) Diabetologia 26, 1178 - 1183.
42. Jenkins, D.J.A., Ghafari, H., Wolever, T.M.S., Taylor, R.H., Jenkins, A.L., Barker, H.M., Fielden, H. and Bowling, A.C. (1982) Diabetologia 22, 450 - 455.
43. Heaton, K.W. (1980) Food intake regulation and fiber. In: Medical Aspects of Dietary Fiber-topics in gastroenterology. (G.A. Spiller and R. Mc Pherson Kay, eds.) Plenum Medical Book Company, New York and London, pp 223 - 238.
44. Mörmann, J.E. and Mühlemann, H.R. (1981) Caries Res. 15, 166 - 175.
45. Frostell, G. (1972) Swed. Dent. J. 65, 161 - 165.
46. Birkhed, D. and Fuchs, G. (1975) Acta Odont. Scand. 33, 59 - 66.
47. Birkhed, D. and Skude, G. (1978) Scand. J. Dent. Res. 86, 248 - 258.
48. Eliasson, A.-C. (1980) Stärke 32, 270 - 272.
49. Rabe, E., Seiler, K. and Gödecke, U. (1980) Zucker u Sussewarenwirtschaft. 33, 158 - 162.
50. Mercier, C. (1980) Structure and digestibility alterations of cereal starches by twin-screw extrusion cooking. In: Food Processing Engineering. Vol I. Food Processing Systems (P. Linko, Y. Mälkki, J. Olkku and J. Larinkari, eds.) Applied Science Publishers Ltd., London, pp 795 - 807.

51. Colonna, P., Melcion, J.-P., Vergnes, B. and Mercier, C. (1983) J. Cereal Sci. 1, 115 - 125.
52. Burt, D.J. and Russel, P.L. (1983) Stärke 35, 354 - 360.
53. Lawton, B.T., Henderson, G.A. and Derlatka, E.J. (1972) Can. J. Chem. Eng. 50, 168 - 172.
54. Mercier, C. (1977) Stärke 29, 48 - 52.
55. Chiang, B.-Y. and Johnson, J.A. (1977) Cereal Chem. 54, 436 - 443.
56. Sahagun, J.F. and Harper, J.M. (1980) J. Food Process Eng. 3, 199 -216.
57. Owusu-Ansah, J., van de Voort, F.R. and Stanley, D.W. (1983) Cereal Chem. 60, 319 - 324.
58. Theander, O. and Westerlund, E. (1983) Chemical modification of starch by heat treatment and further reactions of the products formed. Abstract. COST 91. Concluding Seminar "Thermal Processing and Quality of Foods", Athens 14 - 18 November, Greece.
59. Dahlqvist, A. (1961) Biochem. J. 80, 547 - 551.
60. Dahlqvist, A. (1962) Scand. J. Clin. Lab. Inv. 14, 145 - 151.
61. Mc Neill, J.W., Potter, G.D., Riggs, J.K. and Rooney, L.W. (1975) J. Animal Sci. 40, 335 - 341.
62. Hagqvist, A., Björck, I. and Olkku, J. (1984) Functional properties of extruded wheat products (to be published).
63. Eliasson, A.-C. (1983) Physical properties of starch in concentrated systems such as dough and bread. Dissertation, Dept. Food Technol., University of Lund, Sweden.
64. Holm, J., Björck, I., Ostrowska, S., Eliasson, A.-C., Asp, N.-G., Larsson, K. and Lundquist, I. (1983) Stärke 35, 294 - 297.
65. Mercier, C., Charbonnière, R., Grebaut, J. and de la Guérivière, J.F. (1980) Cereal Chem. 57, 4 - 9.

66. Holm, J., Björck, I., Asp, N.-G. and Lundquist, I. (1984) Physical properties and in vitro/in vivo digestibility of starch after steaming, popping, drum-drying, flaking and extrusion cooking of wheat (to be published).
67. Colonna, P., Doublier, J.L., Melcion, J.P., de Monredon, F. and Mercier, C. (1983) Physical and functional properties of wheat starch after extrusion cooking and drum-drying. Abstract. COST 91. Concluding Seminar "Thermal Processing and Quality of Foods", Athens 14 - 18 November, Greece.
68. Harper, J.M. (1983) Recent applications and research perspectives in the field of extrusion cooking. Abstract. COST 91. Concluding Seminar "Thermal Processing and Quality of Foods", Athens 14 - 18 November, Greece.
69. Anderson, R.A., Conway, H.F., Pfeifer, V.F. and Griffin, E.L. (1969) Cereal Sci. Today 14, 4 - 7, 11.
70. Frostell, G. (1970) Acta Odont. Scand. 28, 599 - 608.
71. Birkhed, D. and Edwardsson, S. (1978). In: Health and Sugar Substitutes. Proceeding of ERGOB Conference, Geneva, Basel, pp 211 - 217.
72. Rerup, C. and Lundquist, I. (1966) Acta Endocrinol. (Kbh) 52, 357 - 367.
73. Bruss, M.L. and Black, A.L (1978) Analyt. Biochem. 84, 309 - 312.
74. Heding, L.G. (1966) A simplified insulin radioimmunoassay method. In: Labelled Proteins in Tracer Studies (L. Donato, G. Milhaud and J. Sirchis, eds.) Euratom, Brussels, pp 345 - 350.
75. Coburn, H.J. and Carrol, J.J. (1973) Clin. Chem. 19, 127.
76. Heding, L. (1975) Diabetologia 11, 541 - 548.
77. Kuzio, M., Dryburgh, J.R., Malloy, K.M. and Brow, J.C. (1974) Gastroenterology 66, 357 - 364.

78. Faloona, G.R. and Unger, R.H. (1974) Glucagon. In: Methods of Hormone Radioimmuno Assay. (B.M. Jasse and H.R. Behrman, eds.) Academic Press, New York, pp 317 - 330.
79. Varriano-Marston, E., Huang, V.K.E.G. and Ponte, J. (1980) Cereal Chem. 57, 242 - 248.
80. Vahouny, G.V. and Kritchevsky, D. (eds.) (1982) Dietary Fibre in Health and Disease, Plenum Press, New York and London.
81. Theander, O. (1983) Advances in the chemical characterisation and analytical determination of dietary fibre components. In: Dietary Fibre. (G.G. Birch and K.J. Parker, eds.), Applied Science Publishers, London and New York, pp 77 - 93.
82. Cummings, J.H. (1982) Consequences of the metabolism of fibre in the human large intestine. In: Dietary Fibre in Health and Disease (G.V. Vahouny and D. Kritchevsky, eds), Plenum Press, New York and London.
83. Brodribb, A.J.M. and Groves, C. (1978) Gut 19, 60 - 63.
84. Heller, S.N., Hackler, L.R., Rivers, J.M., van Soest, P.J., Roe, D.A., Lewis, B.A. and Robertson, J. (1980) Am. J. Clin. Nutr. 33, 1734 - 1744.
85. Wyman, J.B., Heaton, K.W., Manning, A.P. and Wicks, A.C.B. (1976) Am. J. Clin. Nutr. 29, 1474 - 1479.
86. Thomas, B. and Elchazly, M. (1976) Qual. Plant.-Pl. Fds. Hum. Nutr. XXVI, 1, 211 - 226.
87. Asp, N.-G., Johansson, C.-G., Hallmer, H. and Siljeström, M. (1983) J. Agric. Food Chem. 31, 476 - 482.
88. Johansson, C.-G., Siljeström, M. and Asp, N.-G. (1984) Dietary fibre in bread and corresponding flours - Formation of resistant starch during baking. Lebensm. Unters. - Forsch. (submitted).
89. Varo, P., Laine, R. and Koivistoinen, P. (1983) J. Assoc. Off. Anal. Chem. 66, 933 - 938.

90. Asp, N.-G. and Björck, I. (1983) The effect of extrusion cooking on nutritional value. Abstract. COST 91. Concluding Seminar "Thermal Processing and Quality of Foods", Athens 14 - 18 November, Greece.
91. Nyman, M. and Asp, N.-G. (1982) Br. J. Nutr. 47, 357 - 366.
92. Theander, O. and Åman, P. (1979) Swed. J. Agric. Res. 9, 97 - 106.
93. Cummings, J.H., Southgate, D.A.T., Branch, W.J., Wiggings, H.S., Houston, H., Jenkins, D.J.A., Jivraj, T. and Hill, M.J. (1979) Brit. J. Nutr. 41, 477 - 485.
94. Sandberg, A.-S., Anderson, H., Nävert, B. and Sandström, B. (1983) Påverkar extrudering digestionen av stärkelse, kostfiber och fytinsyra i mage och tarm? Abstract. Läkaresällskapets Riksstämma, 30 nov - 2 dec, Stockholm, Sweden.
95. Anderson, J.H., Levine, A.S. and Levitt, M.D. (1981) New Engl. J. Med. 304, 891 - 892.
96. Stephen, A.M., Haddad, A.C. and Phillips, S.F. (1983) Gastroenterology 85, 589 - 595.

Under mild extrusion conditions, wheat products of high protein nutritional value can be obtained. The lysine retention was found to be highly dependent on the process conditions used. An increase in extrusion temperature or screw speed increased lysine damage, whereas an increase in feed rate or moisture content had the opposite effect.

Extrusion cooking of wheat products rendered the starch more susceptible to salivary α -amylase in vitro than did boiling or drum-drying. Consequently, the pH drop in human dental plaque was slightly more pronounced after a mouth rinse with suspensions of extruded products.

The rate of starch absorption in vivo, as judged by the glucose and insulin responses in rats, was similar with boiled wheat products and products extruded under mild conditions. However, under more severe extrusion conditions, there was a significant increase in plasma glucose response, indicating a higher rate of digestion and absorption. In contrast, the plasma glucose and insulin responses to suspensions of drum-dried wheat flour were significantly lower than with a boiled control.

The post-prandial glucose and insulin responses in non-insulin-dependent diabetics were followed after a composite breakfast containing extruded or baked bread. Extruded whole-grain crispbread gave significantly larger areas under the glucose and insulin curves compared with the corresponding baked bread.

There was a small increase in the content of dietary fibre during extrusion cooking of whole-grain wheat flour, and in wheat flour processed under severe extrusion conditions. In extruded wheat flours a significant increase in the proportion of soluble dietary fibre was observed. Studies also showed that extrusion cooking of wheat flour increased the availability of dietary fibre for fermentation in the rat intestinal tract. No such effect was seen in extruded whole-grain flour.

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PROTEIN NUTRITIONAL VALUE OF A BISCUIT

PROCESSED BY EXTRUSION COOKING:

EFFECTS ON AVAILABLE LYSINE

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Protein Nutritional Value of a Biscuit Processed by Extrusion Cooking: Effects on Available Lysine

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The aim of this study was to measure the effect of extrusion cooking on available lysine. The product studied was a protein-enriched biscuit. Available lysine was analyzed chemically as fluorodinitrobenzene (FDNB)-reactive lysine and biologically with growing rats. A comparison was also made with total lysine assayed after conventional acid hydrolysis. The decrease in lysine during extrusion cooking, calculated as percentage of the content in the raw material, was similar with all methods under mild process conditions, about 11%. However, at more severe process conditions the biological assay showed a more pronounced decrease than the chemical methods. Lysine retention was negatively influenced by increased process temperature and positively by increased moisture content of feed. In addition to the lysine loss, there was also a decrease in sulfur-containing amino acids, arginine, and tryptophan. Extrusion cooking under conditions giving a realistic product appears to affect available lysine similarly to, e.g., baking.

From a technological point of view, extrusion cooking of food can be classified as a high-temperature, short-time (HTST) treatment. The process is mainly used for pre-cooking and/or texturization. A variety of products, e.g., crisp bread, biscuits, breakfast cereals, weaning foods, full fat soy flour, and snacks, are processed in extruders, and new applications are made currently.

As the process is under development regarding applications and equipment, and as the nutritional value is of great importance in some of the products, it is essential to study the effect of different processing parameters on the nutritional value.

In the extruder the product is subjected to intense mechanical shear through the action of one or two rotating screws. The cooking takes place at high temperature, high pressure, and a comparatively low water content.

The Maillard reaction is known to be enhanced by high temperatures and intermediate water contents, such as occur during extrusion cooking. Lysine is particularly interesting as it is essential to man and the most reactive protein-bound amino acid, due to its free ϵ -amino group.

The effect of extrusion processing (Brabender-Plasticorder, PL-V500) of lysine-fortified rice was studied by Tsao (1976). The available lysine content, analyzed by the 2,4,6-trinitrobenzenesulfonic acid (TNBS) method (Ousterhout and Wood, 1970), was 89–97% of that in the unprocessed material. In a study by Noguchi et al. (1982) available lysine was analyzed as 1-fluoro-2,4-dinitrobenzene (FDNB)-reactive lysine according to Booth (1971). The product, a protein-enriched biscuit, was processed in a Creusot-Loire BC 45 extruder at different process conditions, and the retention of lysine ranged from 60 to 100%. Pongor and Mátrai (1976) processed soybeans in a Brady 400 type extruder. The available lysine content did not change up to a process temperature of 144 °C but decreased 20–30% at temperatures around 150 °C. Available lysine was measured as the difference between total lysine and residual lysine after treatment with FDNB. Beaufrand et al. (1978) studied the effect of extrusion cooking

(Creusot-Loire BC 45) on lysine in relation to the composition of the raw material. The availability of lysine measured with the FDNB method by difference decreased considerably when sucrose was replaced with fructose, a reducing carbohydrate.

Depending on the kind of Maillard products formed, the different chemical methods (FDNB, TNBS, total lysine) can be expected to correlate more or less well with biological assays. It has been shown that the TNBS method grossly overestimates biologically available lysine in the presence of early Maillard products. Thus, formyl-fructosyllysine that is biologically unavailable was 80% "available" with this method. The FDNB method, on the other hand, showed only 15% availability for this compound, when determined as reactive lysine, while the FDNB method by difference showed 53% availability (Hurrell and Carpenter, 1981). Erbersdobler et al. (1982) compared the reliability of different methods for determination of available lysine in heated protein/sugar mixtures. The FDNB method gave the best correlation with *in vivo* determinations.

The main purpose of the present investigation was to compare the FDNB method for determination of available lysine with a biological assay in extruded products. A Creusot-Loire BC 45 twin screw extruder was used. This equipment has a great versatility regarding applications.

MATERIALS AND METHODS

Chemical Analysis. Nitrogen was determined by the method of Kjeldahl. Amino acids were analyzed after acid hydrolysis (6 N HCl, 110 °C, 24 h) by ion-exchange chromatography. Sulfur-containing amino acids were determined after performic acid oxidation. The equipment used was a Durrum D-500. Tryptophan was analyzed spectrofluorometrically after incubation with papain in urea (Öste et al., 1976). When the chemical score was calculated, the FAO provisional amino acid scoring pattern was used (FAO/WHO, 1973). Available lysine was analyzed as FDNB-reactive lysine as described by Booth (1971) with a slight modification according to Noguchi et al. (1982).

Biological Assay of Available Lysine. Biologically available lysine was estimated from nitrogen balance experiments with growing rats in the following way: Each diet was tested on five male rats (Sprague-Dawley, 75 g). Each rat received 150 mg of nitrogen/day, corresponding to 9.4% protein in the diet. After a 4-day equilibrium period a nitrogen balance was performed for 5 days. Urine and feces were collected separately and analyzed for nitrogen. Fecal "metabolic" nitrogen excretion and urinary

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Table I. Conditions of Extrusion Cooking

sample	mois- ture content of feed, %	feed rate, kg wet wt/h	screw speed, rpm	mass ^d temp, °C	pressure, ^a bars	minimum residence time, s
I	13	40	80	170	195 ~ 200	42
II	13	40	80	193	150 ~ 160	42
III	13	40	80	210	95 ~ 105	44
IV	18	40	80	210	75 ~ 85	44

^a Mass temperature and pressure measured just before the die.

"endogenous" nitrogen were determined with a diet containing 4.5% protein from diethyl ether extracted hen's egg. Calculations of true digestibility (TD), biological value (BV), and net protein utilization (NPU) were performed with the Thomas-Mitchell equations as described by Eg-gum (1973).

The diets were supplemented with minerals, vitamins, choline chloride, and corn oil in optimal amounts for growth as described earlier (Burvall et al., 1977). Cellulose powder, 5%, was added as bulking agent and sucrose, 10%, to improve the edibility of the diet. Corn starch was used to adjust the dry matter content.

Lysine was made limiting in the diets by mixing the sample under investigation with wheat gluten (BDH Chemicals, Ltd.) in the proportions 68:32 regarding nitrogen from the sample and wheat gluten, respectively.

Extrusion Processing. A mixture containing 22% protein was processed in a Creusot-Loire BC 45 twin screw extruder. The protein fraction consisted of 26% wheat protein, 25% casein, and 49% soy protein. The composition of the raw material was as follows: 42% wheat flour (5.5% ash content); 20% corn starch (Fleurine, Corn Products, USA); 20% sucrose (finely ground); 11% soy protein isolate (Promine D, Central Soya); 6% sodium caseinate (low viscosity, Francexpa, 75 Paris); 1% NaCl. The blend was thoroughly mixed for 30 min to get an even distribution of ingredients before processing (M 20-G Lödige mixer, Paderborn, West Germany). The product was a protein-enriched, cereal-based biscuit with the shape of an expanded ribbon.

Product temperature and pressure were measured in the compression chamber just before the die, with a TPT 463 E Dynisco probe (Westwood, MA). The product was processed under four different conditions as shown in Table I. Mass temperature was 170–210 °C just before the die. The moisture content of feed was generally 13% or 18% in one experiment. When the water content was increased from 13 to 18%, it was necessary to increase the temperature of external heaters in order to keep the same product temperature just before the die.

Minimum residence time was determined by using erythrosin and was found to be close to 42–44 s. The screw diameter was 44.2 mm and the screws consisted of five segments (FCCRCRD) (feed CCCRC die; C = compression, R = reverse flighted). The die was rectangular and had an height of 1.5 mm, a width of 22.25 mm, and a length of 47 mm.

The product processed under the mildest conditions was slightly yellowish and can be considered a realistic biscuit. The biscuit processed at 210 °C was brown and must be considered overprocessed. All samples were packed in aluminum-polyethylene bags under vacuum and stored at -20 °C until analysis. Before chemical analysis and biological evaluation, the samples were ground to pass a 0.5-mm screen.

Table II. Amino Acid Composition of Raw Material and Corresponding Extruded Samples (I–IV)

sample	g/16 g of N ^a				
	raw material	I	II	III	IV
isoleucine	4.7	4.6	4.7	4.5	4.5
leucine	8.4	8.1	8.2	8.2	8.2
total lysine	6.3	5.4	4.9	3.9	4.5
FDNB-lysine	5.8	5.4	4.9	3.7	3.9
methionine	2.1	1.8	1.6	1.5	1.5
cystine	1.4	1.3	1.2	1.2	1.2
phenylalanine	5.2	4.7	4.6	5.0	4.9
tyrosine	4.1	3.7	3.6	3.8	3.7
threonine	4.1	3.8	3.9	3.9	4.0
tryptophan ^c	1.7	1.7	1.6	1.5	1.5
valine	5.3	5.0	5.1	5.1	5.0
aspartic acid	9.3	9.1	9.2	8.9	9.0
serine	6.3	5.7	5.7	6.0	6.0
glutamic acid	24.0	24.0	24.0	23.1	23.4
proline	8.4	8.1	8.1	8.7	8.4
glycine	3.7	3.5	3.3	3.6	3.5
alanine	3.9	3.6	3.7	3.9	3.8
histidine	2.7	2.6	2.5	2.5	2.5
arginine	6.1	6.1	5.0	4.8	5.1

^a The figures are mean values from duplicates. ^b Per-formic acid oxidation. ^c Spectrofluorometrical assay.

The biscuits extruded under the four different conditions will be referred to as samples I–IV. The mixture of ingredients before extrusion will be referred to as raw material.

RESULTS

Amino Acids. The amino acid contents are shown in Table II. There was a substantial decrease in total lysine with increasing process temperature. The loss was 13% in the mildest processed sample (I) and 37% in the most severely processed one (III). An increased water content had a clearly beneficial effect on the lysine retention. There was also a decrease in sulfur-containing amino acids, in arginine, and in tryptophan. After lysine the loss in methionine was most pronounced, amounting to 26–28% in the samples processed under the most severe conditions. Cystine decreased about 17% and the loss in arginine was up to 20%. The tryptophan content did not change at the lower temperature, but at 210 °C the loss was 10%. The other analyzed amino acids did not change significantly.

FDNB-Reactive Lysine. The decrease in FDNB-reactive lysine was moderate in the sample processed at 170 °C, about 7%. At 210 °C the decrease was substantial, about 37%. At this temperature, an increase in water content from 13 to 18% H₂O gave a higher retention of FDNB-lysine (Table II).

Biological Assay of Available Lysine. In the raw material calculation of the chemical score (c.s.) showed that the sulfur-containing amino acids were limiting. In order to make lysine limiting in all the test diets, wheat gluten was added. On the basis of the amino acid analysis, a diet was chosen with 68% of its nitrogen coming from the extruded samples or from the raw material and 32% from gluten. As there was also a decrease in the sulfur-containing amino acids during extrusion, all diets were supplemented with L-methionine (0.4 g/16 g of N). For the raw material and sample (I) extruded at the lowest temperature, the score for threonine and valine was very close to that of lysine after mixing with gluten. Those amino acids were therefore also added to all diets, 0.4 and 0.3 g/16 g of N, respectively.

Chemical scores for the diets are given in Table III. Lysine was limiting and the range of c.s. was 59–88%. As a control c.s. was calculated also from data of the re-

Table III. Chemical Score of the Sample/Gluten Mixtures

chemical score	raw material/ gluten ^d	sample I/ gluten ^d	sample II/ gluten ^d	sample III/ gluten ^d	sample IV/ gluten ^d
isoleucine	109	108	108	106	106
leucine	113	111	111	111	112
lysine	88	78	71	59	67
methionine + cystine	116 ^a	107 ^a	103 ^a	101 ^a	101 ^a
phenylalanine + tyrosine	149	138	135	143	141
threonine	101 ^b	97 ^b	98 ^b	99 ^b	100 ^b
tryptophan	139	144	136	128	127
valine	104 ^c	100 ^c	100 ^c	101 ^c	100 ^c

^a 0.36 g of L-methionine/16 g of N added. ^b 0.38 g of L-threonine/16 g of N added. ^c 0.29 g of L-valine/16 g of N added. ^d N of sample/N of gluten = 68/32.

Table IV. True Digestibility (TD), Biological Value (BV), and Net Protein Utilization (NPU) of the Sample/Gluten Mixtures

	TD ± SD	BV ± SD	NPU ± SD
raw material/gluten ^a	96.9 ± 0.9	73.4 ± 1.8	71.1 ± 2.0
sample I/gluten ^a	96.8 ± 1.2	69.2 ± 2.0	67.0 ± 2.0
sample II/gluten ^a	95.5 ± 0.9	62.8 ± 3.0	59.9 ± 3.3
sample III/gluten ^a	92.7 ± 1.1	55.0 ± 2.2	51.0 ± 2.4
sample IV/gluten ^a	94.6 ± 1.3	61.1 ± 1.7	57.7 ± 1.9

^a N of sample/N of gluten = 68/32.

quirements of the growing rat given by NAS/NRC (1972). Those data also include histidine and arginine. Lysine was found to be clearly limiting in all diets also according to these calculations.

Table IV shows the results of the nitrogen balance experiments. Since the wheat gluten used is completely digestible, the digestibility changes demonstrated correspond to a decrease from about 95% in the raw material and the mildest extruded sample (I) to about 89% in the most severely extruded one (III). In sample IV, processed with higher water content, the digestibility change was less pronounced. The biological value of the sample/gluten mixture decreased from 73% in the mixture with the raw material to 55% in the mixture with sample III, extruded at 210 °C. Again the sample processed at the same temperature but at a higher water content (IV) was less affected.

In order to get a quantitative measure of biologically available lysine, a second experiment was performed, in which free amino acids were added to wheat gluten in amounts equal to the amino acid content in the raw material. The ratio of "sample" nitrogen to gluten nitrogen was the same as in the first experiment. The lysine content was varied at the expense of alanine to keep total nitrogen constant in all mixtures. The chemical score for lysine was varied in the interval 50–88% to make a standard curve that would fit the samples. The full composition of amino acids in the standard mixtures is given in Table V.

The results from the nitrogen balance study performed on these standard mixtures are given in Table VI. The BV and NPU (almost identical in this experiment because of the TD close to 100%) can be expressed as a function of "added" lysine as shown in Figure 1. A linear correlation was obtained with the correlation coefficient of 0.99.

Comparison of FDNB-Reactive Lysine, Total Lysine, and Biologically Available Lysine. Available lysine measured biologically, BV = *f* (available lysine), was consistently lower than FDNB-lysine (Table VII). In the raw material and in the sample extruded at higher water

Table V. Amino Acid Composition of the Standard Diets

	g/16 g of N		
	gluten	free amino acids	c.s. of mixture ^a
isoleucine	1.2	3.2	108
leucine	2.3	5.7	
lysine	0.6	2.2–4.2	50–88
methionine	0.6	1.8	
cystine	0.8	1.0	116
phenylalanine	1.6	3.5	
tyrosine	1.1	2.8	147
threonine	0.9	3.2	101
tryptophan ^c	0.2	1.1	139
valine	1.3	3.9	104
aspartic acid	1.1	6.3	
serine	1.8	4.3	
glutamic acid	11.1	16.2	
proline	3.8	5.7	
glycine	1.0	2.5	
alanine	0.9	4.7–2.7	
histidine	0.7	1.8	
arginine	1.2	4.2	

^a N of sample/N of gluten = 68/32. ^b Performic acid oxidation. ^c Spectrofluorometrical assay.

Table VI. True Digestibility (TD), Biological Value (BV), and Net Protein Utilization (NPU) of the Standard Diets

added lysine, g/16 g of N	TD ± SD	BV ± SD	NPU ± SD
2.18	100.9 ± 0.8	54.6 ± 1.7	55.1 ± 2.0
2.73	100.6 ± 0.3	61.9 ± 1.2	62.2 ± 1.3
3.28	100.3 ± 0.6	69.2 ± 0.7	69.5 ± 0.9
3.82	100.3 ± 0.7	76.4 ± 1.2	76.6 ± 0.8
4.23	100.7 ± 0.5	77.7 ± 2.2	78.2 ± 2.5

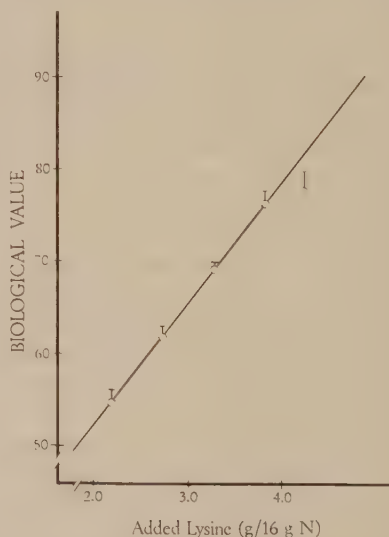


Figure 1. Biological value as a function of lysine added to the standard diets.

content (18%), the results were in good agreement, however, the methods differing only by 5 and 3%, respectively. For the other extruded samples the difference was 10–20% (Figure 2). When the calculation of biologically available lysine is based on NPU, taking into account also the digestibility, the overestimation of available lysine with the FDNB method is more pronounced (Table VII).

Table VII. Effects of Extrusion Cooking on Lysine: Comparison between Total Lysine, FDNB-Reactive Lysine, and a Biological Determination Performed on Rats

	lysine, g/16 g of N			
	total	FDNB reactive	biologically available	
			BV	NPU
raw material	6.3 (100) ^a	5.8 (100)	5.5 (100)	5.1 (100)
extruded samples				
I (170 °C, 13% H ₂ O, 80 rpm)	5.4 (87)	5.4 (93)	4.8 (88)	4.5 (87)
II (193 °C, 13% H ₂ O, 80 rpm)	4.9 (77)	4.9 (85)	4.0 (73)	3.6 (70)
III (210 °C, 13% H ₂ O, 80 rpm)	3.9 (63)	3.7 (63)	3.1 (56)	2.6 (50)
IV (210 °C, 18% H ₂ O, 80 rpm)	4.5 (72)	3.9 (68)	3.8 (70)	3.4 (65)

^a Values in parentheses are percent.

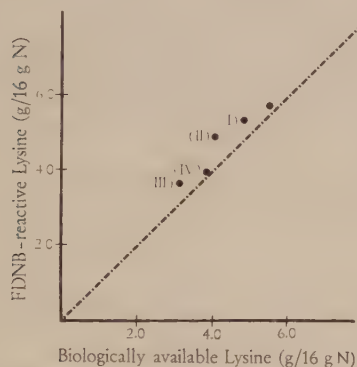


Figure 2. FDNB-reactive lysine as a function of biologically available lysine calculated from the biological value. The extruded samples are indicated (I-IV).

Total lysine after acid hydrolysis was in general higher than both FDNB-reactive lysine and biologically available lysine, as shown in Table VII.

The decrease in lysine during extrusion cooking calculated as a percentage of the lysine content in the raw material was similar for total lysine and for available lysine calculated from the BV. FDNB-reactive lysine showed a slightly lower relative decrease for samples extruded under the milder conditions (I and II). The decrease in available lysine calculated from NPU was more pronounced under the more severe process conditions, due to the decreased digestibility.

DISCUSSION

Determination of FDNB-reactive lysine or other *in vitro* methods does not reflect changes in the protein digestibility. Lysine with a free ϵ -amino group may be physiologically unavailable if adjacent peptide bonds are not split in the digestive tract. This may, therefore, cause an error in the chemical determination of available lysine in samples with a decreased protein digestibility.

An objection to the biological assay is that the standard curve was based on free amino acids. The rate of digestion and absorption can be expected to differ from that of the test protein (Mathews and Adibi, 1976). Nitrogen balance experiments in which mixtures of free amino acids have been compared with the corresponding proteins show divergent results, however. Amino acid mixtures corresponding to casein and egg albumin gave BV and NPU similar to the proteins. For soy protein the NPU and BV for the corresponding amino acid mixtures were significantly higher than for the protein, whereas in the case of gluten NPU was higher and the BV lower for the amino acid mixture (Forsum and Hambraeus, 1977). In our study the test protein was a mixture of casein, soy, and wheat protein. Thus, it is not possible to predict whether our

biological assay would give a negative or a positive error, if any. In severely processed samples toxic derivatives, if produced, could influence the biological responses. This is, however, an objection to all biological measurements.

The standard curve $BV = f(\text{available lysine})$ is not valid for $BV < 40$. At such low levels of lysine the curve is not linear (Bender, 1961).

Under the process conditions used in the present study, lysine was damaged at the fastest rate. The lysine retention was highly dependent on the process temperature and on the water content of the feed. Other amino acids—cystine, arginine, and tryptophan—known to participate in the Maillard reaction (Hurrell and Carpenter, 1977) were also affected. In addition to losses in lysine, Beaufrand et al. (1978) reported significant losses of arginine and histidine during extrusion cooking in a Creusot-Loire BC 45 extruder.

The decrease in total methionine in our study is more difficult to interpret as the thioether group does not normally combine with reducing sugars (Cuq et al., 1978).

The reason for reduced availability of protein-bound methionine during heat treatment of proteins is not fully known, although a decreased protein digestibility and oxidation of methionine residues are believed to be important. During heat treatment methionine may be oxidized to methionine sulfoxide and methionine sulfone. Methionine sulfone is unavailable whereas methionine sulfoxide is partly available at least in the rat (Cheftel, 1979). Available methionine can decrease considerably during heat treatment (Rao and Mc Laughlan, 1967).

Pongor and Mátrai (1976) studied the changes in methionine during extrusion cooking of soybean in a single screw extruder. Unaltered methionine, that is, free methionine recovered after acid hydrolysis, was used as a measure of availability. Corrections were made to compensate for destruction of methionine during hydrolysis. At a process temperature of 149 °C, the decrease in methionine was 10%. Total methionine measured after oxidation to sulfone did not change, however.

As total methionine normally is determined as methionine sulfone, it is not likely that oxidation during processing has any great impact on the result. To account for the prominent loss in the samples processed at the most severe conditions in our study, methionine must have been altered in such a way as to make oxidation to sulfone impossible. However, losses in methionine could not be found in the most severely extruded sample (sample III) when analyzed directly without prior oxidation to methionine sulfone. This discrepancy deserves further investigation.

Losses in total methionine has been reported in the literature (Miller et al., 1965a). In a study by Donoso et al. (1962) heat treatment of pork resulted in a 16% loss in total methionine. Miller et al. (1965b) studied different methods for determination of total methionine and con-

cluded that analysis of methionine sulfone could give divergent results in heated materials. That is, a decrease in total methionine after heat treatment could be due to analytical artifacts.

The raw material used in our study contained only small amounts of reducing sugars initially. The reason for the considerable loss in lysine at the higher temperatures is probably production of reducing monosaccharides through hydrolysis of sucrose. This has been reported to take place during extrusion cooking by Noguchi et al. (1982), and these authors suggested that the Maillard reaction might be limited by the formation of reducing sugars through sucrose hydrolysis. Dextrinization of starch has also been reported (Tsao, 1976; Mercier, 1977; Sahagun and Harper, 1980), which could further aggravate lysine loss.

Under the mildest conditions chosen in our study (170 °C, 13% H₂O, 80 rpm) the lysine loss was about 11% regardless of analytical method. This loss is close to that reported during bread baking (Rosenburg and Rohdenburg, 1951). The similar decrease in total and available lysine at mild conditions in our study indicates that the mechanism of lysine damage is destruction rather than reduced availability. This is in agreement with results found during high-temperature, short-time baking of pizza crust (Tsen et al., 1982a) and balady bread (Tsen et al., 1982b). However, under more severe extrusion conditions (210 °C, 13%, H₂O, 80 rpm) there is a discrepancy between the chemical methods and the biological assay (37 vs. 50% lysine loss). This discrepancy was mainly due to a reduction in the protein digestibility. The product processed at this high temperature was dark brown in color and cannot be considered a realistic product. Losses of this magnitude (50%), however, have been reported when baking protein-enriched biscuits containing skim milk powder (Carpenter and March, 1961). When conventional baking, microwave baking, and steaming were compared, conventional baking significantly reduced the availability of lysine (Tsen et al., 1977). However, only minor differences were seen in total lysine between the three methods of baking. Thus, depending on the food process and/or processing temperature, it seems as if the nature of lysine damage may vary.

For practical purposes determination of total lysine after acid hydrolysis or of FDNB-reactive lysine seems useful to predict deterioration of protein nutritional value during extrusion cooking, when expressed relative to the content in the raw material.

Registry No. Lysine, 56-87-1; arginine, 74-79-3; tryptophan, 73-22-3.

LITERATURE CITED

- Beaufrand, M. J.; de la Guerivière, J. F.; Monnier, C.; Poullain, B. *Ann. Nutr. Aliment.* **1978**, *32*, 353-364.
- Bender, A. E. *Publ. Nat. Res. Council.* **1961**, No. 843.
- Booth, V. H. *J. Sci. Food. Agric.* **1971**, *22*, 658-666.
- Burvall, A.; Asp, N.-G.; Dahlqvist, A.; Öste, R. *J. Dairy Res.* **1977**, *44*, 549-553.
- Carpenter, K. J.; March, B. E. *Br. J. Nutr.* **1961**, *15*, 403-410.
- Cheftel, J. C. "Nutritional and Safety Aspects of Food Processing"; Marcel Dekker: New York and Basel, 1979; Chapter 6.
- Cuq, J. L.; Aymard, C.; Besancon, P.; Cheftel, J. C. *Ann. Nutr. Aliment.* **1978**, *32*, 307.
- Donoso, G.; Lewis, A. M.; Miller, D. S.; Payne, P. R. *J. Sci. Food Agric.* **1962**, *13*, 192-196.
- Eggum, B. O. "406 Beretning fra forsøgslaboratoriet. A study of certain factors influencing protein utilization in rats and pigs"; Statens Husdyrbrugsudvalg: København, Denmark, 1973.
- Erbersdobler, H. F.; Andersson, T. R.; Holstein, A.-B. "Book of Abstracts", 183rd National Meeting of the American Chemical Society, Las Vegas, NV, March 1982; American Chemical Society: Washington, DC, 1982; AGFD 97.
- FAO/WHO W. H. O. *Tech. Rep. Ser.* **1973**, No. 522.
- Forsum, E.; Hambraeus, L. *Naeringsforskning* **1977**, *21*, 90.
- Hurrell, R. F.; Carpenter, K. J., presented at the IUFOST Symposium, Norway, 1977, No. 27.
- Hurrell, R. F.; Carpenter, K. J. *Prog. Food Nutr. Sci.* **1981**, *99*, 159-176.
- Mathews, D. M.; Adibi, S. A. *Gastroenterology* **1976**, *71*, 151-161.
- Mercier, C. *Staerke* **1977**, *28*, 48-52.
- Miller, E. L.; Carpenter, K. J.; Milner, C. K. *Br. J. Nutr.* **1965a**, *19*, 547-564.
- Miller, E. L.; Hartley, A. W.; Thomas, D. C. *Br. J. Nutr.* **1965b**, *19*, 565-573.
- NAS/NRC "Nutrient Requirement of Laboratory Animals"; NAS/NRC: Washington, DC, 1972; No. 10.
- Noguchi, A.; Mosso, K.; Aymard, C.; Jeunink, I.; Cheftel, J. C. *Lebensm.-Wiss. Technol.* **1982**, *15*, 105-110.
- Öste, R.; Nair, B.; Dahlqvist, A. *J. Agric. Food. Chem.* **1976**, *24*, 1141-1144.
- Ousterhout, L. E.; Wood, E. M. *Poult. Sci.* **1970**, *49*, 1423.
- Pongor, S.; Mátrai, T. *Acta Aliment. Acad. Sci. Hung.* **1976**, *5*, 49-55.
- Rao, M. N.; Mc Laughlan, J. M. *J. Assoc. Off. Anal. Chem.* **1967**, *50*, 704.
- Rosenburg, H. R.; Rohdenburg, E. L. *J. Nutr.* **1951**, *45*, 593-598.
- Sahagun, J. F.; Harper, J. M. *J. Food Process Eng.* **1980**, *3*, 199-216.
- Tsao, T. Dissertation, Colorado State University, Fort Collins, CO, 1976.
- Tsen, C. C.; Bates, L. S.; Wall, L. L.; Gehrke, C. W. *J. Food Sci.* **1982a**, *47*, 674-675.
- Tsen, C. C.; Reddy, P. R. K.; El-Samahy, S. K.; Gehrke, C. W. "Book of Abstracts", 183rd National Meeting of the American Chemical Society, Las Vegas, NV, March 1982; American Chemical Society: Washington, DC, 1982b; AGFD 94.
- Tsen, C. C.; Reddy, P. R. K.; Gehrke, C. W. *J. Food Sci.* **1977**, *42*, 402-406.

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II

PROTEIN NUTRITIONAL VALUE OF EXTRUSION-COOKED WHEAT FLOURS

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Food Chemistry (accepted for publication)

ABSTRACT

The effect of extrusion cooking on the protein nutritional value of wheat flour and whole-grain wheat flour was studied. Amino-acid analysis showed lysine retention between 63 and 100%. The retention was positively affected by an increase in feed rate, and negatively by an increase in screw speed.

The prominent lysine damage at severe conditions was probably due to formation of reducing carbohydrates through hydrolysis of starch. The loss of other amino acids was small. The BV of extruded wheat flours was significantly reduced in the products showing the most prominent lysine loss. However, no effect was seen in TD. In contrast, a decrease in BV of extruded whole-grain flour was accompanied by a significant reduction in TD. Extrusion cooking of wheat starch did not affect the nitrogen balance in rats given casein/starch diets, containing raw or extruded starch.

INTRODUCTION

Most studies on the protein nutritional value of extruded products have been performed on blended foods processed in single-screw extruders (Jansen et al., 1978; Harper & Jansen, 1981; Del Valle et al., 1981). Less biological data are available on twin-screw extruders, which are able to operate over a wider range of moisture contents.

The main mechanism responsible for protein deterioration during processing is the Maillard reaction or non enzymic browning. In a previous study on the protein quality of an extruded cereal based biscuit containing sucrose, a significant decrease in the availability of lysine, as well as in protein digestibility, was observed at severe conditions (Björck et al., 1983). It has been suggested that the rate of the Maillard reaction, during extrusion cooking of such a mixture, is limited by the formation of reducing sugars through hydrolysis of sucrose (Noguchi et al., 1982). In addition, hydrolysis of starch (Kim, 1983; Owusu-Ansah et al., 1983; Theander & Westerlund, 1983), may provide additional reactive carbohydrates.

Unabsorbed carbohydrates have been shown to affect the metabolic and endogenous losses of nitrogen in the rat (Asp & Dahlqvist, 1976; Nyman & Asp, 1982), possibly due to an increased activity of the colonic bacteria. Formation of enzyme resistant starch has been reported during food processing (Theander & Westerlund, 1983; Johansson et al., 1984). Even if only a small fraction of starch is passed undigested to the colon, it would provide the colonic bacteria with a substantial amount of fermentable carbohydrates. This could

have methodological implications when evaluating the protein nutritional value of processed products in nitrogen balance experiments.

The present investigation was performed to study the protein nutritional value of extruded wheat flours and whole-grain wheat flours. The products were processed in a Creusot-Loire BC 45 twin-screw extruder under different conditions, without additional carbohydrates present. The amino acid composition was determined by gas-liquid chromatography, and the degree of browning by reflectance measurements. Protein digestibility and biological value was evaluated through nitrogen balance studies in rats.

MATERIALS AND METHODS

Materials

Commercially available wheat flour (80% extraction rate), whole-grain wheat flour (Vaasa Mills Ltd, Finland) and wheat starch (A-starch, Raisio Factories Ltd, Finland) was used in the experiments. The materials were extruded in a Creusot-Loire BC 45 twin-screw extruder under different conditions as described in **Table 1**. The extruded products and the whole-grain wheat flour were ground to pass a 0.5 mm sieve.

Chemical analysis

Nitrogen content was determined by the Kjeldahl procedure. Amino acids were analyzed by gas-liquid chromatography as N-trifluoroacetyl-n-butyl derivatives after acid hydrolysis of the protein and subsequent derivatization (Nair, 1977). Colour was measured with a Elrepho 21000 reflectance photometer. The sample cup was filled with approximately 30 ccm, and the reflectance was measured at 577 nm with an opal glass standard.

Animal experiments

The nitrogen balance experiments were performed in young rats (Sprague Dawley, 75 g) according to Eggum (1973). Each diet was tested on five animals. Food intake was restricted to 10 g dry matter per day, containing 150 mg N.

In the case of wheat flour and whole-grain wheat flour, the flour itself was used as the protein source. In the groups fed raw or extruded wheat starch 69.3% (dry basis), the protein source was casein (ANRC reference protein, ICN Nutritional Biochemicals) supplemented with 0.2% l-methionine (BDH). All diets contained 5% corn oil, 4.8% minerals and 1% vitamins, including choline chloride. The mineral and vitamin mixtures were as described previously (Björck et al., 1984). The sucrose content in the diets was varied to adjust dry matter. Faecal metabolic and urinary endogenous excretion was determined with a diet containing 4.5% protein from diethyl ether extracted hen's egg. After a four-day adaption period, a nitrogen balance was performed for five days. Urine and faeces were collected separately in metabolic cages, and the content of nitrogen was analyzed. Faeces was collected dry, removed every day and frozen at -20°C . The faeces was then lyophilized, weighed and milled to pass a 0.4 mm screen, and stored at -20°C until analysis. Calculation of true digestibility (TD), Biological Value (BV) and Net Protein Utilization (NPU) was performed with the Thomas-Mitchells equations as described by Eggum (1973).

RESULTS AND DISCUSSION

The amino acid composition before and after extrusion cooking of wheat flour is shown in **Table II**. The lysine loss ranged from 0 to 37% depending on process conditions. No significant losses occurred in other amino acids. The most prominent lysine loss (37%) occurred at the highest mass temperature WF4. An increase in screw speed from 150 rpm (WF3) to 200 rpm (WF4) at otherwise similar conditions, increased the lysine loss from 25 to 37% ($P < 0.05$). In contrast, an increase in feed rate, from 200 g/min (WF5) to 350 g/min (WF1), significantly improved the lysine retention ($P < 0.01$). No lysine loss was observed in product WF1, whereas the loss was considerable, 25%, in the product processed at a lower feed rate.

The amino acid composition in the whole-grain wheat flour products was almost unaffected by processing (**Table III**), except at the most severe conditions used (WGF3), where 9% of the lysine was lost.

As judged from the amino acid composition in the raw materials, milling of wheat flour caused a decrease in the content of especially lysine and aspartic acid, whereas the opposite was found for glutamic acid and proline (**Table II and III**). This is in agreement with other reports on the effect of milling on the amino acid pattern (Pedersen & Eggum, 1983).

In **Fig 1**, reflectance has been plotted versus lysine content in the different extruded wheat flour products. Evidently, there is a correlation between the degree of browning and the lysine content in processed samples ($r=0.95$). However, the reflectance obtained with raw flour (88.3) deviated considerably from all processed samples, including that with no lysine loss. This was probably due to other factors than Maillard products affecting the reflectance, e.g. starch gelatinization.

Results from nitrogen balance experiments with the wheat flour products and the most severely processed whole-grain product are shown in **Table IV**. With wheat flour, true protein digestibility (TD) was unaffected by processing even at severe conditions. In contrast, with whole-grain wheat flour (WGF3), there was a significant decrease in TD (6%) during processing. At mild conditions, the biological value (BV) of the wheat flour products was similar to that of the raw wheat flour, about 50, whereas the BV and the net protein utilization (NPU) of product WF3-WF5 was significantly reduced. In the whole-grain product, there was also a significant decrease in BV and NPU. As lysine is the limiting amino acid in wheat, a diminished availability in processed products will be reflected by a decrease in BV. However, with lysine limiting at or below maintenance, the BV levels off at about 40 due to for instance endogenous synthesis or reduced catabolism (Bender, 1961).

As judged from the weight gains (close to zero) of the rats given the most severely processed products (WF3-WF5), the lysine content in these products was at the maintenance level. This could explain why the decrease in BV was less pronounced than the decrease in total lysine in these products. Consequently, although the lysine content in product WF4 was significantly different from product WF5. ($P<0.01$) and WF3 ($P<0.05$), no significant difference was observed in BV. However, an increase in feed rate from 200 g/min (WF5) to 350 g/min (WF1) significantly improved the BV ($P<0.01$). Due to a higher lysine content in whole-grain wheat, the decrease in BV and lysine in product WGF3 was similar, about 10%.

Results from balance experiments with casein/starch diets, containing raw or extruded wheat starch, are shown in **Table V**. No effect was seen in any of the parameters TD, BV or NPU due to extrusion cooking of the starch. Thus, the losses of nitrogen in the urine and faeces were not affected, indicating that insignificant amounts of undigestible starch were formed during processing of pure starch.

Rats given the most severely processed wheat flour (WF4) developed diarrhoea, making it difficult to separate urine and faeces in the metabolic cages. This may have affected the TD and BV, but not the NPU of this product. In this case, faeces was collected in 5% H_2SO_4 as described by Eggum (1973). The stools of rats given product WF3 and WF5 were slightly watery but did not present any problems during collection. These effects of severely processed wheat flour on faeces cannot be explained at present.

The loss of lysine in the extruded whole-grain wheat flours was much less pronounced than in the wheat flour products. This was probably due to a more narrow temperature interval and a higher moisture content of feed during processing of whole-grain wheat. The beneficial effect of a higher moisture content on lysine retention, has been demonstrated previously (Noguchi et al., 1982; Björck et al., 1983). The lysine loss in whole-grain wheat was accompanied by a decrease in TD, whereas no effect was seen on TD in the wheat flour products. This observation is difficult to interpret. However, it has been suggested that the intense mixing during extrusion cooking may reduce the protein digestibility due to interactions between protein and undigestible polysaccharides present in the cell-walls (Linko et al., 1981). It is notable that the BV of the most severely processed whole-grain flour was still similar to that of unprocessed wheat flour.

Very little attention has been paid to the impact of feed rate on product characteristics (Linko et al., 1981). In the present study, it was shown that an increase in feed rate significantly improved the protein nutritional value of the product. This is probably a result of a shorter residence time.

Conflicting results exist on the impact of screw speed on the protein nutritional value (Tsao, 1976; Noguchi et al., 1982). An increase in screw speed at otherwise similar conditions in our study, increased the lysine loss. This could not be verified in the biological assay, as the lysine loss was too prominent in these products. Although the lysine destruction was much more pronounced when fructose, a reducing carbohydrate, was added during extrusion cooking of a cereal based mixture, the loss was considerable (30%) also with only starch present (Beaufrand et al., 1978). An increase in shear rate has been reported to promote dextrinization of starch (Sahagun & Harper, 1980), thus providing reducing carbohydrates that can participate in the Maillard reaction (Racicot et al., 1981). According to Andersson et al. (1981), an increase in screw speed during extrusion cooking of a starch/gluten/bran mixture led to an increase in burnt taste, which may correspond to a higher level of Maillard compounds.

Colour measurements are easy to perform, and can provide information about the extent of lysine damage in processed products. In a study by Lorenz et al. (1974), an increase in extrusion temperature during processing of triticale increased product colour, whereas an increase in moisture content had the opposite effect. In this study, a correlation was found

between colour and lysine content in extruded wheat products. However, the colour measurements could not be used to predict the lysine loss relative the raw material.

In conclusion, under carefully selected extrusion conditions, wheat products with high protein quality can be obtained. However, although only minute amounts of reducing sugars were present originally in the raw flours, the lysine loss was considerable (37%) at severe process conditions, possibly due to formation of reducing carbohydrates through hydrolysis of starch.

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REFERENCES

- Andersson, Y., Hedlund, B., Jonsson, L. & Svensson, S. (1981).
Extrusion cooking of a high-fiber cereal product with crispbread
character, Cereal Chem. **58**, 370-4.
- Asp, N.-G. & Dahlqvist, A. (1976). Inverkan av laktos på kvävebalansen
hos råtta. Näringsforskning **20**, 49-50.
- Beaufrand, M.J., de la Guérivière, J.V., Monnier, C. & Poullain, B. (1978).
Influence du procédé du cuisson extrusion sur la disponibilité des
protéines. Ann. Nutr. Alim., **32**, 353-64.
- Bender, A.E. (1961). In: Progress in meeting protein needs of infants and
preschool children, Proc. symp. (L. Voris, (Ed.)) Publs. natn. Res. Counc.,
Wash. no. 843, pp. 407-25.
- Björck, I., Noguchi, A., Asp, N.-G., Cheftel, J.C. & Dahlqvist, A. (1983).
Protein nutritional value of a biscuit processed by extrusion cooking:
Effects on available lysine. J. Agric. Food Chem. **31**, 488-92.
- Björck, I., Nyman, M. & Asp, N.-G. (1984). Extrusion cooking and dietary
fiber - Effects on dietary fiber content and on degradation in the rat
intestinal tract. Cereal Chem. (in press)
- Del Valle, F.R., Villanueva, H., Reyes-Govea, J., Escobedo, M., Bourges, H.,
Ponce, J. & Munoz, M.J. (1981). Development, evaluation and industrial
production of a powdered soy-oats infant formula using a low-cost
extruder. J. Food Sci., **46**, 792-7.
- Eggum, B.O. (1973). A study of certain factors influencing protein utilization
in rats and pigs. 406 Beretning fra forsøgslaboratoriet. Statens Husdyr-
brugsudvalg, Dissertation, Copenhagen, Denmark.
- Harper, J.M. & Jansen, G.R. (1981). Nutritious foods produced by low-cost
technology. LEC Report 10, Colorado State University, Fort Collins, Colorado.

- Jansen, G.R., Harper, J.M. & O'Deen, L. (1978). Nutritional evaluation of blended foods made with a low-cost extruder cooker. J. Food Sci. **43**, 912-5, 925.
- Johansson, C.-G., Siljeström, M. & Asp, N.-G. (1984). Dietary fibre in bread and corresponding flours - Formation of resistant starch during baking. Ztscht für Lebensmittel-Unters. & -Forschg (submitted).
- Kim, J.C. (1983). Effect of some extrusion parameters on the solubility and viscograms of extruded wheat flour. Paper presented at COST 91 Concluding Seminar "Thermal processing and quality of foods", Athens, 14-18 November, 1983.
- Linko, P., Colonna, P. & Mercier, C. (1981). High-temperature, short-time extrusion cooking. In: Advances in cereal science and technology (Pomeranz, Y. (Ed.)), American Association of Cereal Chemists Incorporated, St. Paul, Minnesota, pp. 145-235.
- Lorenz, K., Welsh, J., Hormann, R., Beetner, G. & Frey, A. (1974). Extrusion processing of triticale. J. Food Sci., **39**, 572-6.
- Nair, B.M. (1977). Gas-liquid chromatographic analysis of amino acids in food samples. J. Agric. Food Chem., **25**, 614-20.
- Noguchi, A., Mosso, K., Aymard, C., Jeunink, J. & Cheftel, J.C. (1982). Maillard reactions during extrusion-cooking of protein-enriched biscuits. Lebensm. Wiss. u. -Technol., **15**, 105-10.
- Nyman, M. & Asp, N.-G. (1982). Fermentation of dietary fibre components in the rat intestinal tract. Br. J. Nutr., **47**, 357-66.
- Owusu-Ansah, J., van de Voort, F.R., Stanley, D.W. (1983). Physiochemical changes in corn starch as a function of extrusion variables. Cereal Chem., **60**, 319-24.
- Pedersen, B. & Eggum, B.O. (1983). The influence of milling on the nutritive value of flour from cereal grains. 2. Wheat. Qual. Plant Foods Hum. Nutr., **33**, 51-61.

- Racicot, W.F., Satterlee, L.D. & Hanna, M.A. (1981). Interaction of lactose and sucrose with corn meal proteins during extrusion. J. Food Sci., **46**, 1500-6.
- Sahagun, J.F. & Harper, J.M. (1980). Effects of screw restrictions on the performance of an autogenous extruder. J. Food Proc. Eng., **3**, 199-216.
- Theander, O. & Westerlund, E. (1983). Chemical modification of starch by heat treatment and further reactions of the products formed. Poster presented at COST 91 Concluding Seminar "Thermal processing and quality of foods", Athens, 14-18 November, 1983.
- Tsao, T.-F. (1976). Available lysine retention during extrusion processing. PhD Dissertation, Colorado State University, Fort Collins, Colorado.

TABLE 1

Process Conditions^a During Extrusion Cooking of Wheat Flour, Whole Grain Wheat Flour and Wheat Starch

	Wheat flour					Whole grain flour			Starch
	WF1	WF2	WF3	WF4	WF5	WGF1	WGF2	WGF3	
Mass feed rate including moisture (g/min)	350	200	200	200	200	200	200	200	200
Feed moisture content (%)	15	15	15	15	15	20	20	20	15
Screw speed (rpm)	150	100	150	200	150	100	150	200	200
Mass temperature ^b (°C)	161	161	171	171	156	164	166	166	180

^a Creusot-Loire BC 45 twin screw extruder. Set temperature of barrel 150°C. Die: diameter 5 mm; capillary length 27 mm; clearance die/end plate 2 mm. Screw: combination Die-RCCCC-feed: R, Reverse flight screw element; C Compression screw element; co-rotating intermeshing screws.

^b Mass temperature was measured with a thermocouple inserted just before the die.

TABLE II

Amino Acid Composition in Raw and Extrusion Cooked Wheat Flour

Amino acid (g/16 g N)	Raw material	Extrusion cooked materials				
		WF1 (%)	WF2 (%)	WF3 (%)	WF4 (%)	WF5 (%)
Iso-Leucine	4.4	98	100	102	100	98
Leucine	8.5	99	99	101	95	98
Lysine	2.4	100	96	75***	63***	75***
Phenylalanine	5.2	96	94	88	104	96
Threonine	3.2	94	100	97	88	91
Valine	5.3	96	102	109	94	98
Alanine	4.1	90	100	102	107	93
Glycine	4.0	100	105	105	103	105
Proline	14.5	97	97	94	91	94
Serine	5.4	106	91	85	92	98
Aspartic acid	4.6	91	107	93	91	96
Glutamic acid	36.7	100	-	-	-	101

Significantly different from the corresponding raw material

*** P<0.001 (Students' t-test)

TABLE III

Amino Acid Composition in Raw and Extrusion Cooked Whole Grain Wheat Flour

Amino acid (g/16 g N)	Raw material	Extrusion cooked materials		
		WGF1 (%)	WGF2 (%)	WGF3 (%)
Iso-Leucine	3.8	111	105	105
Leucine	7.5	103	101	104
Lysine	3.2	106	94	91 [*]
Phenylalanine	4.8	92	102	110
Threonine	3.1	113	107	107
Valine	4.7	102	104	113
Alanine	4.3	105	102	105
Glycine	4.4	107	102	107
Proline	11.1	110	103	106
Serine	4.9	104	99	101
Aspartic acid	5.8	107	0	96
Glutamic acid	30.3	108	100	104

Significantly different from the corresponding raw material

^{*} P<0.05 (Students' t-test)

TABLE IV

True Digestibility (TD), Biological Value (BV) and Net Protein Utilization (NPU) of
Raw and Extrusion Cooked Wheat Samples

	TD [±] SD	BV [±] SD	NPU [±] SD
<u>Wheat flour</u>			
Raw	94.4 [±] 2.6	50.7 [±] 3.8	47.8 [±] 3.6
WF1	96.3 [±] 0.9	50.6 [±] 1.7	48.7 [±] 1.7
WF2	95.5 [±] 0.7	48.7 [±] 2.0	46.5 [±] 1.8
WF3	93.9 [±] 2.1	40.2 [±] 2.3 (***) ^a	37.8 [±] 2.6 (***) ^a
WF4	93.5 [±] 3.0	37.6 [±] 5.1 (***) ^a	35.3 [±] 5.8 (***) ^a
WF5	95.5 [±] 1.3	40.8 [±] 3.0 (***) ^a	39.0 [±] 3.2 (***) ^a
<u>Whole grain wheat flour</u>			
Raw	89.0 [±] 1.9	55.8 [±] 2.4	49.6 [±] 2.5
WGF 3	84.4 [±] 1.7(**) ^a	49.7 [±] 2.9 (**) ^a	41.9 [±] 1.7 (***) ^a

^a Significantly different from the corresponding raw material

** P<0.01, *** P<0.001 (Student's t-test)

TABLE V

True Digestibility (TD), Biological Value (BV) and Net Protein Utilization (NPU) of Casein with Raw or Extrusion Cooked Wheat Starch as Source of Carbohydrate

	TD [±] SD	BV [±] SD	NPU [±] SD
Casein ^a + Raw wheat starch	100.5 [±] 1.6	92.1 [±] 1.3	92.5 [±] 2.4
Casein ^a + Extrusion cooked wheat starch	99.8 [±] 1.5	93.4 [±] 2.1	93.2 [±] 3.1

^a The diet was supplemented with 0.2% L-methionine (dry basis)

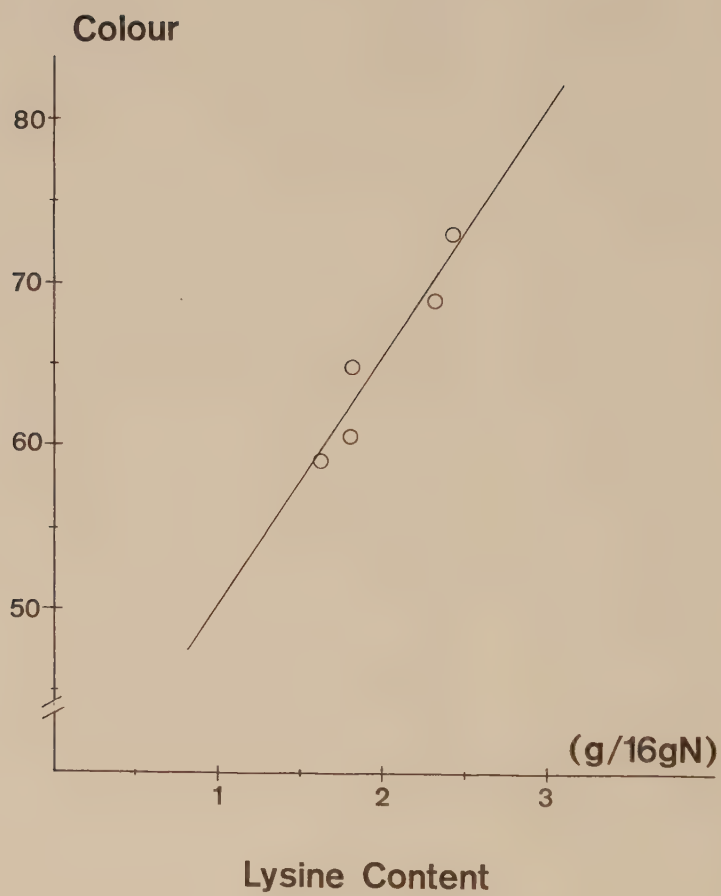


Fig 1. Product colour as a function of the lysine content

III

EFFECTS OF PROCESSING ON STARCH AVAILABILITY

IN-VITRO AND IN-VIVO :

EXTRUSION COOKING OF WHEAT FLOURS AND STARCH

I. Björck, N.-G. Asp, D. Birkhed & I. Lundquist

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Effects of Processing on Starch Availability *In Vitro* and *In Vivo*-Extrusion Cooking of Wheat Flours and Starch

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The effect of extrusion cooking on starch availability in wheat products was tested *in vitro* and *in vivo*. Hydrolysis *in vitro* was measured using salivary amylase and the *in vivo* availability was evaluated by measuring the plasma levels of glucose and insulin after a gastric load in conscious rats. The fermentability of starch in human dental plaque *in situ* was also studied. Extrusion cooking of white flour and whole-grain wheat flour rendered the starch more susceptible to salivary α -amylase than did boiling for 20 min in water. Consequently, the pH drop in dental plaque was more pronounced with extruded materials. The rate of starch absorption *in vivo*, as judged from the plasma glucose pattern, was similar for boiled samples and for samples extruded under mild conditions. However, under more severe extrusion conditions, there was a significant increase in plasma glucose response. In the case of white flour, this enhanced glucose response was accompanied by an increased insulin response. Thus, processing conditions can influence both the glycaemic response to starch and its cariogenic properties.

Introduction

Recent studies have indicated that different starchy foods influence the post-prandial glucose and insulin responses differently^{1,2}. Soluble pure starch is hydrolyzed very rapidly *in vivo*, rendering the absorption at the brush-border level the rate-limiting step³. However, when present in a food matrix, not all starches are utilized at the same rate, indicating that intraluminal digestion might be rate-limiting. Cooking and gelatinization of starch, is known to increase its susceptibility to amylase. Apart from affecting the substrate, heat-treatment also inactivates α -amylase inhibitors present in some foods, e.g. in raw cereals. Processing may also affect the availability of carbohydrates by mechanically disrupting the structure of the material, thus increasing the surface area/starch ratio⁴.

The rate of carbohydrate absorption from the intestine is of interest in relation to diabetes and obesity. Slowly-absorbed carbohydrates are considered to be beneficial in the regulation of diabetes, and can also be expected to favour a longer duration of satiety after a meal in healthy subjects. Owing to the presence of α -amylase in saliva, an increased susceptibility of starch to hydrolysis in the oral cavity may also enhance the development of dental caries.

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The extent to which different thermal treatments, used in gelatinization and pre-cooking, affect the availability of starch, is not well documented. High-temperature, short-time (HTST) extrusion cooking is a comparatively new process that is now used increasingly in the food industry for cooking and texturization, mainly for cereal-based products. A number of products are produced in extrusion cookers, e.g. gruels, breakfast cereals, crispbreads, biscuits, and further new applications are being introduced. Extrusion cooking takes place at elevated temperature (100–250 °C) and pressure (2–20 MPa), and at low water contents (10–30%). Normally the residence time is in the order of 5 to 120 s. During cooking, the material is subjected to intense mechanical treatment through the action of one or two rotating screws. The screw flights transport and mix the ingredients (powdery or granular) to a uniform dough-like mass, which is pressed through a die.

The die is designed to produce the desired shape and the product can be used either directly or after being ground to a powder. Technical reviews on extrusion cooking have been published recently^{5, 6}. The minimum amount of water necessary for gelatinization of wheat- and potato-starch under normal conditions (atmospheric pressure, low shear rates, etc) is about 30%⁷. However, during extrusion cooking a complete gelatinization of cereal starches can be obtained at lower water contents, i.e. 16–22%⁸.

The purpose of the present investigation was to study the effect of extrusion cooking on the availability of starch to physiological enzymes. Extrusion-cooked wheat starch, white flour and whole-grain wheat flour were compared with the raw materials and with raw materials that had been boiled in water at atmospheric pressure for 20 min. Starch availability was tested *in vitro* with salivary α -amylase and *in vivo* by measuring the plasma glucose and insulin responses after a gastric load in young rats. At the end of the experiment liver glycogen levels were determined. The effect of processing on cariogenicity was evaluated by measuring the pH changes of human dental plaque *in situ*.

Experimental

Commercially-available wheat starch (A-starch, Raisio Factories Ltd, Finland), white flour from wheat and whole-grain wheat flour (Vaasa Mills Ltd, Finland) were used in the experiments. The materials were extruded in a Creusot-Loire BC 45 twin-screw extruder under different conditions as described below. The screw combination and die geometry were kept constant (die diameter 5 mm, capillary length 27 mm, clearance between die and end plate, 2 mm). Processing conditions were as given in Table I.

Preparation of samples

The extrusion-cooked materials, which had the shape of expanded ribbons, and the whole-grain wheat flour were ground to pass a 0.5 mm sieve. This was carried out in order to permit comparison with the raw material and with materials that had been gelatinized conventionally by boiling in water. Suspensions were prepared by adding an amount of sample, equivalent to 5 g starch (dry matter), to 80 ml water. The raw materials were boiled in a water bath for 20 min with gentle stirring. All suspensions were homogenized for a few seconds in an Ultra-Turrax homogenizer and were diluted either with K^+ - Na^+ -phosphate buffer for *in vitro* tests or with NaCl solution for *in vivo* experiments in rats as described below.

TABLE 1. Process conditions in the Creusot-Loire BC 45 twin screw extruder

Sample code*	Wheat starch				White flour					Whole-grain wheat flour		
	S1	S2	S3	WF1	WF2	WF3	WF4	WF5	WGF1	WGF2	WGF3	
Mass temperature (°C)†	158	176	180	161	161	171	171	156	164	166	166	
Feed moisture content (% w/w)	20	15	15	15	15	15	15	15	20	20	20	
Feed rate (g/min)	200	200	200	350	200	200	200	200	200	200	200	
Screw speed (r/min)‡	100	150	200	150	100	150	200	150	100	150	200	

* See text for details.

† Barrel temperature set at 150 °C.

‡ Screw combination: Feed-CCCCR-Die where C and R represent compression and reverse flight screw elements, respectively.

Starch analysis

All samples were heated in a boiling water bath for 15 min before analysis. Starch was analyzed after incubation with amyloglucosidase (Boehringer, Mannheim, W. Germany), and liberated glucose was quantified with a glucose oxidase reagent (Glox Novum, Kabi Diagnostica, Sweden)⁹. Starch contents were calculated as glucose $\times 0.9$. Soluble starch 'nach Zulkowsky' was used as reference (Merck, Darmstadt, W. Germany). This reference material contained 80% starch (wet basis), corresponding to approximately 96% recovery of starch (dry, ash-free basis).

Starch hydrolysis *in vitro* using salivary amylase

A stock solution of salivary amylase was prepared by diluting saliva (1 ml) with 50% aqueous glycerol (40 ml). Before collecting the saliva, the donor's mouth was rinsed thoroughly with water. Amylase activity was measured at 37 °C with soluble starch 'nach Zulkowsky' as substrate. A 0.05 M K⁺-Na⁺-phosphate buffer, pH 6.9, containing 0.04% (w/v) NaCl was used to produce optimal conditions for the enzyme ($K_m = 8 \cdot 10^{-4}$ g/ml starch). The stock solution of salivary amylase was adjusted to contain 17 International Units (IU) of activity/ml (one IU catalyses the liberation of 1 μ mol maltose/min at 37 °C). Hydrolysis *in vitro* of the different materials was measured by incubating 10 ml substrate, equivalent to 20 mg starch (dry matter), with 10 ml of a 1:20 dilution of the stock salivary amylase solution. All solutions were prepared with K⁺-Na⁺-phosphate buffer and incubation was performed at 37 °C. Samples (2 ml) of the incubation mixture were withdrawn at suitable time intervals and mixed with dinitrosalicylic acid (DNS) reagent (2 ml)¹⁰, which inactivated the enzyme. Maltose was used as a standard, and the degree of hydrolysis (%) was calculated as $100 \times (\text{mg maltose equivalent} / \text{mg starch} \times 1.05)$. Soluble starch 'nach Zulkowsky' was used in each experiment as a control.

Paper chromatography

After incubating the sample with salivary amylase as above for 3 h, the amylase was inactivated by boiling for 10 min. The incubation mixture contained 20 mg starch (dry matter) and the total volume was 20 ml. Products formed during enzymic hydrolysis *in vitro* were extracted with 80% (v/v) ethanol-H₂O for 1 h. Insoluble material was removed by filtration and washed with 80% ethanol. The filtrate was concentrated by evaporation and then diluted with distilled H₂O to a final volume of 5 ml. Samples (20 μ l) of this test solution were applied to Whatman no. 3 paper. The chromatograms were developed with a solvent comprising ethyl acetate:acetic acid:H₂O (3:1:1 by vol.). The papers were dipped in silver nitrate reagent and developed with ethanolic NaOH¹¹. The ultimate products from hydrolysis of starch by salivary amylase are maltose, maltotriose, α -limit dextrins and small amounts of glucose¹². In order to evaluate the extent of hydrolysis, glucose, maltose, and two low-mol. wt dextrins, panose and maltopentaose were used as markers (50 μ g).

Experiments *in vivo* on young rats

Male rats (Sprague-Dawley) weighing 150–170 g were used. The animals were fasted for 16 h before the experiments but they were allowed free access to water. From 10 to 20 rats were used in each group. The rats were given extruded white flour (WF2, WF4) or extruded whole-grain wheat flour (WGF1, WGF3) processed under different conditions. Water absorption index was measured as described by Anderson *et al.*¹³. The flours extruded under mild conditions, WF2 and WGF1, had water absorption indices suitable for production of cereal-based weaning foods, such as corn-soya-milk, (5.0 and 4.8 respectively). Judging by the water absorption index and the colour, the flours extruded under more severe conditions, WF4 and WGF3, must be considered to be overcooked. The water absorption indices for these materials were 2.9 and 3.7 respectively. Apart

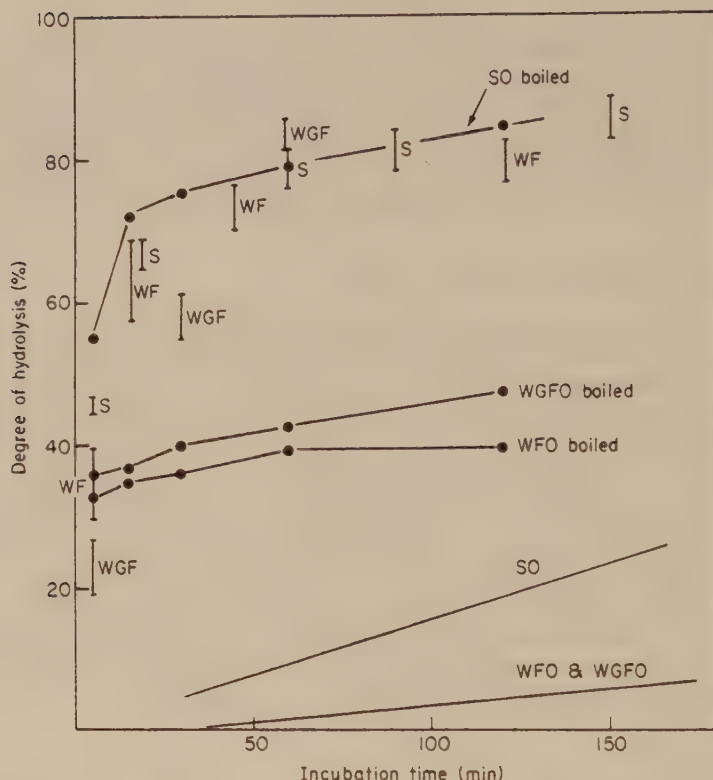


FIGURE 1. Starch hydrolysis with salivary amylase *in vitro*. The same starch concentration was used with all samples. The degree of hydrolysis is expressed as maltose equivalents. Due to low levels of hydrolysis, no experimental points are given for raw materials (SO, WFO and WGFO), experimental points with boiled materials are marked (●). The bars (I) represents the interval obtained with extruded, materials. *Wheat starch*: raw (SO), boiled (SO boiled) and extrusion cooked S1–S3 (S); *White flour*: raw (WFO), boiled (WFO boiled) and extrusion cooked WF1–WF5 (WF); *Whole-grain wheat flour*: raw (WGFO), boiled (WGFO boiled) and extrusion cooked WGF1–WGF3 (WGF).

from a higher process temperature, these products were processed at a much higher screw speed. Process conditions for the different samples are given in Table I. Flour that had been gelatinized by boiling for 20 min in water was used as a control.

Test suspensions were prepared from the different wheat samples by adding 0.9% NaCl to give a final concentration of 50 mg starch/ml. The suspensions were tempered in a water bath to 37 °C and then kept at this temperature during feeding. Aliquots of these suspensions (2 ml), corresponding to 100 mg starch (dry basis), were given through a stomach tube. Blood samples were taken at suitable time intervals after intubation. Determination of plasma glucose concentration was carried out with a glucose oxidase method¹⁴ and plasma insulin was analyzed with a radio-immunoassay¹⁵. The insulin values were expressed in International Units as μU ($25 \mu\text{U} \equiv 1 \text{ ng insulin}$). The blood sampling procedure was performed on conscious animals by puncture of the retroorbital sinus as described previously¹⁶. After 120 min the rats were killed by a blow to the head and a liver specimen (c. 0.5 g) was taken from the ventral part of the left lateral lobe for glycogen analysis. Liver glycogen was determined by the method of Rerup and Lundquist¹⁷.

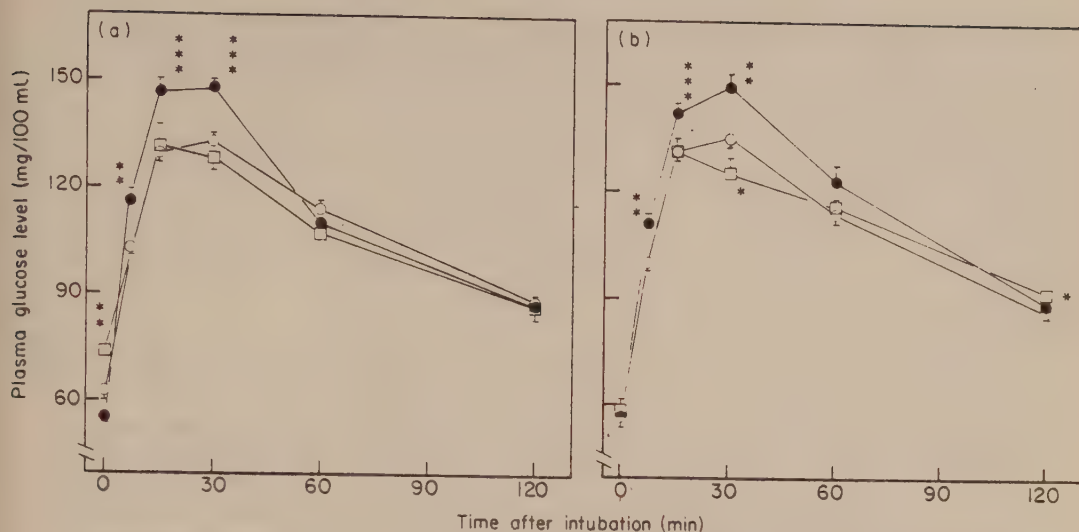


FIGURE 2. Plasma glucose levels in young rats following a gastric load containing 100 mg starch as a 5%, w/v, suspension in 0.9% NaCl. Ten to 20 rats in each group. Mean values $\pm$ SEM. Statistical evaluation: Student's *t* test—**P* < 0.05, ***P* < 0.01 and ****P* < 0.001. (a) *White flour*: boiled (○); extrusion cooked, mild conditions – WF2 (□) and severe conditions – WF4 (●). (b) *Whole-grain wheat flour*: boiled (○); extrusion cooked, mild conditions – WGF1 (□) and severe conditions – WGF3 (●).

Changes in pH of dental plaque

Eighteen subjects were instructed not to clean their teeth for two days before the experiment and not to eat or drink on the morning of the examination. On the morning of the third day, the subjects rinsed their mouth with tap water to remove loose debris before rinsing for 30 s with the test suspension (10 ml). The following test suspensions, containing an equivalent amount of carbohydrate (5% w/v), were used: glucose, soluble stable 'nach Zulkowsky' boiled for 20 min, raw white flour, white flour boiled for 20 min and extrusion-cooked white flour (samples WF2 and WF4).

Samples of dental plaque were taken for pH determination immediately before (0 min plaque pH) and at 2, 5, 10, 20 and 30 min after rinsing¹⁸. The different test suspensions were administered to the subjects in random order with an interval of one week between tests.

Results

Starch availability in vitro

Starch content in the raw material was, on dry basis: 96.5% in wheat starch, 73.0% in white flour and 60.0% in the whole-grain wheat flour. Processing did not alter the starch content significantly in any of the products.

Hydrolysis with salivary α -amylase *in vitro* was very rapid in all extruded samples (Fig. 1). The degree of hydrolysis for starch-, white flour- and whole-grain wheat flour-products are marked as an internal as there was no obvious correlation with extrusion conditions. The degree of hydrolysis reached a plateau at a level corresponding to 80% hydrolysis. White flour, and whole-grain wheat flour that had been gelatinized for 20 min by boiling,

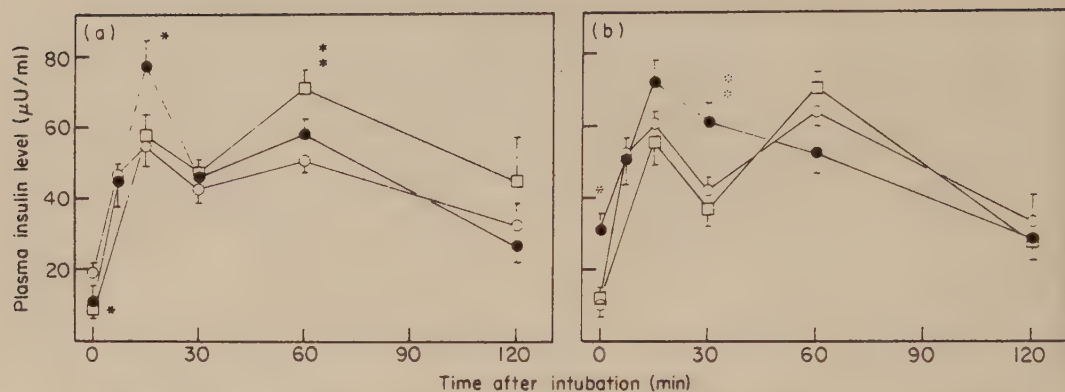


FIGURE 3. Plasma insulin levels in young rats following a gastric load containing 100 mg starch as a 5%, w/v, suspension in 0.9% NaCl. Ten to 20 rats in each group. Mean values $\pm$ SEM. Statistical evaluation: Student's *t* test – **P* < 0.05 and ***P* < 0.01. (a) White flour: boiled (○); extrusion cooked, mild conditions – WF2 (□) and severe conditions – WF4 (●). (b) Whole-grain wheat flour: boiled (○); extrusion cooked, mild conditions – WGF1 (□) and severe conditions – WGF3 (●).

were much less available to the enzyme under the *in vitro* conditions used. On the other hand, boiled wheat starch was hydrolyzed as rapidly as the extruded samples. Raw samples were hydrolyzed very slowly *in vitro*. Autoclaving of wheat flour (20 min, 125 °C) increased starch availability to the same level as with extrusion cooking.

Products formed after 3 h incubation (i.e. after reaching the plateau) were separated by paper chromatography. Extruded materials (WF1 and WF5) gave a higher yield of maltose and a lower yield of higher mol. wt. maltodextrins than the boiled white flour control. These observations are in agreement with the results on the degree of hydrolysis measured with DNS. Glucose could not be detected with any of the three materials studied.

Starch absorption *in vivo* in young rats

To study the importance of these differences *in vitro*, for the uptake of starch *in vivo*, young rats were given suspensions of boiled and extruded flours by gastric intubation. All test suspensions contained an equivalent amount of starch (see Experimental). Plasma glucose and insulin level were measured at intervals over a 2 h period. The results are shown in Figs 2 and 3. Glucose and early insulin responses to flours extruded under mild conditions did not differ significantly from those found with boiled controls. However, both the white flour and the whole-grain wheat flour, extruded under severe conditions, gave a significantly higher glucose response 7, 15 and 30 min after intubation. These was also a tendency to corresponding increases in the early insulin response.

The plasma glucose and insulin responses to boiled white flour and boiled whole-grain wheat flour were very similar (Table II). However, liver glycogen content (mg/g wet weight, mean value of 20 animals $\pm$ SEM) was significantly higher with whole-grain wheat flour, 8.2 ± 0.6 as compared with white flour, 5.8 ± 0.5 (*P* < 0.01). No difference was

TABLE II. Plasma levels of glucose and insulin in young rats following a gastric load containing 100 mg starch as a 5% w/v suspension in 0.9% NaCl. Comparison between boiled white flour (WF) and boiled whole-grain wheat flour (WGF). Mean values of 20 animals $\pm$ SEM.

Time after intubation (min)	Plasma glucose level (mg/100 ml)		Plasma insulin level (μ U/ml)*	
	Boiled WF	Boiled WGF	Boiled WF	Boiled WGF
0	62.9 $\pm$ 2.5	56.7 $\pm$ 2.5	19.3 $\pm$ 2.9	15.1 $\pm$ 3.5
15	129.8 $\pm$ 2.6	131.3 $\pm$ 2.7	54.7 $\pm$ 5.2	60.8 $\pm$ 3.6
30	132.7 $\pm$ 2.4	135.5 $\pm$ 2.6	42.7 $\pm$ 3.6	42.0 $\pm$ 3.7
60	114.1 $\pm$ 2.3	114.2 $\pm$ 2.5	50.8 $\pm$ 3.1	64.2 $\pm$ 4.1†
120	88.8 $\pm$ 1.2	87.4 $\pm$ 1.1	32.7 $\pm$ 6.4	33.2 $\pm$ 7.7

* 25 μ U $\equiv$ 1 ng insulin.

† Significantly higher than with white flour. Student's *t* test $P < 0.05$.

observed in liver glycogen levels between boiled flours and the different extruded samples. However, when the results for extruded whole grain wheat flours were combined to include both extrusion conditions, there was a significant difference compared with the boiled control. Liver glycogen content in rats given extruded whole-grain wheat flour, WGF1 and WGF3, was reduced to 6.7 ± 0.5 mg/g wet weight ($P < 0.05$).

Changes in pH of dental plaques

The effect of rinsing the mouth with the various test suspensions is presented in Fig. 4. Glucose resulted in the lowest plaque pH values, followed by boiled soluble starch and the extrusion-cooked wheat flours (WF2 and WF4). Boiled wheat flour also induced a similar pH drop in the plaque, but the recovery was more rapid than for glucose, extrusion-cooked wheat flours (WF2 and WF4) and boiled soluble starch. Raw wheat flour resulted in less pronounced decreases in pH than the other test suspensions. Student's *t*-test for paired observations was used to determine the statistical significance of differences between the pH values obtained with the test suspensions, or between the decreases relative to the 0 min pH value, at different time intervals¹⁸. The statistical evaluation is presented in Table III. Glucose and raw white flour differed significantly from all other products. The pH decrease after rinsing with extruded products was significantly greater than for the boiled control at several time intervals. No significant difference was observed between WF2 and WF4 or between boiled soluble starch and WF4.

Discussion

Starch content measured with amyloglucosidase was very similar before and after extrusion cooking. This is in agreement with earlier reports on extruded cereal products^{19, 20}. The levelling off at about 45% hydrolysis *in vitro* in the boiled flours is in agreement with results by Jenkins *et al.*² with boiled cereal products. These authors

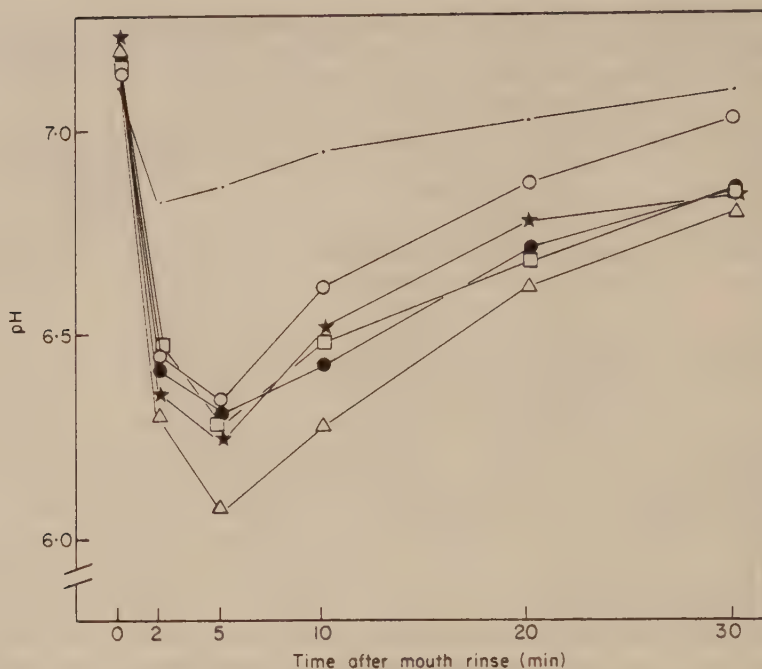


FIGURE 4. Changes in pH of human superficial dental plaque *in situ* after a 30 s-mouth rinse with 10 ml of the following test suspensions (5%, w/v, carbohydrate): glucose (Δ), boiled soluble starch ($\star$), raw white flour ($\cdot$), boiled white flour ($\circ$), extrusion cooked white flours - mild conditions, WF2 ($\square$) and severe conditions, WF4 ($\bullet$). Mean values of 18 subjects.

TABLE III. Significant differences* in dental plaque pH at different time intervals after rinsing with the following test suspensions (5%, w/v carbohydrate): raw wheat flour (WF raw), boiled wheat flour (WF boiled), extrusion cooked wheat flours - mild conditions (WF2) and severe conditions (WF4), boiled soluble starch (Starch) and glucose

	Time after mouth rinsing (min)										
Δ pH	0	2	Δ 0-2	5	Δ 0-5	10	Δ 0-10	20	Δ 0-20	30	Δ 0-30
WF raw-WF boiled		***	***	***	***	**	**	*	*		
WF boiled-WF2								**		*	
WF boiled-WF4						*	*				*
WF2-WF4	—	—	—	—	—	—	—	—	—	—	—
Starch-WF2			*								
Starch-WF4	—	—	—	—	—	—	—	—	—	—	—
Starch-glucose						*					
WF raw-glucose		***	***	***	***	***	***	***	***	**	***
WF boiled-glucose			*	**	**	***	***	***	**	**	***
WF2-glucose			*	*							
WF4-glucose				*	*						

* Student's paired *t* test; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; —, not significant. Calculations performed on 18 subjects.

used salivary amylase in combination with jejunal juice. Under the process conditions used in our study, extrusion cooking gave a higher availability *in vitro* of starch in flours than did boiling. This could be due to the intense mechanical treatment in the extruder, resulting in a uniform and disorganized structure. Extrusion cooking, even if performed at low temperature (about 130 °C), has been reported to result in complete destruction of starch granule structure in wheat^{21, 22}. Furthermore, some dextrinization of cereal starches takes place²³, which may increase the rate of hydrolysis with α -amylase *in vitro*. In contrast to boiled flours, boiled wheat starch was equally available to salivary amylase as the starch in extruded materials.

Particle size, and the degree of separation of bran and endosperm, appears to influence the rate of starch hydrolysis *in vitro*. Boiled, finely-ground, whole-grain wheat flour was more susceptible to α -amylase than boiled, coarsely-ground flour²⁴. Furthermore, reconstituted whole-grain wheat flour was more susceptible to α -amylolysis *in vitro* than was the original whole-grain wheat flour, indicating the importance of the physical state. It has been suggested also that physical separation of starch and protein may increase the digestibility of starch²⁵. The importance of the physical state on hydrolysis *in vitro* has been demonstrated also with extruded materials, in which milling significantly increased the availability to α -amylase. However, under severe extrusion conditions, the availability was *not* improved further by milling²¹.

The finding that boiled starch and various starch products cause lowering of pH in dental plaque is in agreement with earlier reports²⁸⁻²⁹. In the mouth, starch is hydrolyzed mainly in saliva and, to a minor extent, within the plaque itself^{28, 30}. After hydrolysis, maltose, maltotriose and residual low mol. wt. dextrins diffuse into the plaque and are metabolized to organic acids by the microorganisms present. The method used for measuring pH-changes in dental plaque in this study permits only analysis of superficial plaque, collected mainly from buccal and lingual tooth surfaces. Lower pH values are obtained if the pH of interdental plaque is measured^{28, 29}. Regarding the 'cariogenicity' of a certain product, a critical pH value of 5.7 has been accepted from telemetric recordings of interdental plaque *in vivo*²⁹. This corresponds to approximately pH 6.5 with the method used in our study^{29, 31}. Therefore, all test suspensions in our study, with the exception of raw flour, may be considered more or less cariogenic as judged from the pH decrease.

The suspensions differed in the availability of starch to salivary α -amylase *in vitro* and also, as a consequence, in their pH-reducing effect in dental plaque. The results indicate that extrusion cooking, even if performed under mild conditions, makes the starch easily fermentable by microorganisms in the plaque, approximately to the same extent as boiled soluble starch. Therefore, extruded products must be considered cariogenic, especially if they are consumed frequently or if they are retained for long periods in the mouth. It has been shown that the contact time between starch and the saliva is a critical factor for the pH drop in plaque²⁸. In our study the contact time was kept constant by using a 30 s mouth rinse. However, the true contact time may have been longer if adhesion had occurred. Consequently, a reduced stickiness in the extruded products must be considered beneficial in connection with dental health.

In contrast, the correlation between starch availability *in vitro*, and starch absorption *in vivo*, was not so consistent. The higher glycaemic response for the sample extruded

under severe conditions than that for the boiled control is in agreement with the results from hydrolysis *in vitro*. The samples extruded under milder conditions, however, did not differ from the boiled controls *in vivo*, despite the higher susceptibility to amylase *in vitro*. This may indicate either that the efficiency of amylolysis *in vivo* is high enough to eliminate the differences observed *in vitro*, or that other factors, apart from the availability to α -amylase, were of major importance for the rate of absorption *in vivo*.

In other studies on starch availability, good correlation has been observed between *in vitro* and *in vivo* results^{2, 32, 33}. However, raw wheat starch, which is comparatively resistant to enzymic digestion *in vitro*, is, nevertheless, digested *in vivo*. Furthermore, differences in the release of gastrointestinal hormones, in the rate of gastric emptying or in the rate of intestinal absorption, cannot be assessed *in vitro*. The interesting *in vitro* technique developed recently, using physiological enzymes and dialysis, is an attempt to make the *in vitro* assay more comparable to the *in vivo* situation². With this technique it was observed that different foods had different abilities to trap products formed during starch hydrolysis within the dialysis bag. This trapping effect may correspond to a reduced rate of absorption, and is not revealed solely by following the rate of hydrolysis.

In our study, no systematic difference was found *in vitro* between samples extruded under different conditions. However, with rats, the most severely-processed flours gave rise to significantly higher glucose responses than did boiled controls. Thus, it appears that differences in the disruption of starch granules, due to the severity of the extrusion conditions, can give rise to differences in the rates of starch absorption *in vivo*. It is unlikely that the differences in viscosity between the extruded products can explain the increased rate of absorption found with flours extruded under severe conditions, although these were less viscous. In a recent study, glucose and boiled pure starches of different viscosities produced similar serum glucose responses in humans³⁴. Furthermore, even at low levels of hydrolysis, the viscosity of a starch gel is reduced. In man, it has been shown that a substantial amount of the swallowed salivary amylase remains active when passing through the stomach owing to the buffering capacity of the meal³⁵.

The similarities in the glucose and insulin responses to boiled white flour, and to boiled whole-grain wheat flour, indicate that the difference in fibre content (3.9 versus 13.0%, dry basis) did not have any influence on the rate of starch absorption. This is in agreement with other studies in humans showing that wheat bran had only minor effects on post-prandial glycemia when added to a test meal³⁶. In contrast, decreased post-prandial glucose and insulin responses to viscous dietary fibre, such as pectin and guar gum, are well documented^{36, 37}.

The finding that boiled, whole-grain wheat flour produced a significantly higher liver glycogen level than did boiled white flour 2 h after feeding, cannot be explained at present. It has been suggested that the glycogen pool may be important in the regulation of hunger and satiety³⁸. A higher level of liver glycogen after a meal could, therefore, be considered as being beneficial in terms of satiety. The average liver weight of 160 g-rats is close to 6 g. Based on this figure, 35% of the intubated starch from white flour was deposited as glycogen in the liver, compared with about 50% of the starch from whole-grain wheat flour.

In conclusion, extrusion cooking of wheat flour increased the availability of starch to salivary amylase, compared with boiling, as judged by an *in vitro* assay and by the

decrease of pH in dental plaque. Extrusion cooking under mild conditions did not increase the rate of starch absorption in the rat above that for boiled materials: on the other hand, extrusion cooking under higher temperature and shear conditions did increase the rate of starch absorption. In this respect, the effect of different extrusion conditions should be evaluated further and comparisons should be made with alternative processes (e.g. drum-drying, spray-drying, autoclaving and baking).

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References

1. Crapo, P. A. Reaven, G. and Olefsky, J. *Diabetes* **26** (1977) 1178-1183.
2. Jenkins, D. J. A., Ghafari, H., Wolever, T. M. S., Taylor, R. H., Jenkins, A. L., Barker, H. M., Fielden, H. and Bowling, A. C. *Diabetologia* **22** (1982) 450-455.
3. Dahlqvist, A. and Borgström, B. *Biochem. J.* **81** (1961) 411-418.
4. O'Dea, K., Nestrel, P. J. and Antonoff, L. *Am. J. Clin. Nutr.* **33** (1980) 760-765.
5. Harper, J. M. *Extrusion of Foods*, CRC Press, Boca Raton, Florida (1981) vols I and II.
6. Linko, P., Colonna, P. and Mercier, C. High-temperature, short-time extrusion cooking, in 'Advances in Cereal Science and Technology', (Y. Pomeranz, ed.) American Association of Cereal Chemists St. Paul, MN (1981) vol. IV, pp 145-235.
7. Eliasson, A. C. *Die Stärke* **32** (1980) 270-272.
8. Rabe, E., Seiler, K. and Gödecke, U. *Zucker Sussewaren Wirtsch.* **33** (1980) 158-162.
9. Dahlqvist, A. *Biochem. J.* **80** (1961) 547-551.
10. Hostettler, F., Borel, E. and Deuel, H. *Helv. Chim. Acta* **34** (1951) 2132-2139.
11. Trevelyan, W. E., Procter, D. P. and Harrison, J. S. *Nature* **166** (1950) 444-445.
12. Greenwood, C. T. and Milne, E. A. *Stärke* **5** (1968) 139-150.
13. Anderson, R. A., Conway, H. F. and Peplinski, A. J. *Die Stärke* **22** (1970) 130-134.
14. Bruss, M. L. and Black, A. L. *Anal. Biochem.* **84** (1978) 309-312.
15. Heding, L. G. in 'Labelled Proteins in Tracer Studies' (L. Donato *et al.*, eds), Euratom, Brussels (1966) pp 345-350.
16. Rerup, C. and Lundquist, I. *Acta Endocrinol. (Kbh)* **52** (1966) 357-367.
17. Rerup, C. and Lundquist, I. *Acta Pharmacol. Toxicol.* **25** (1967) 41-53.
18. Frostell, G. *Acta Odont. Scand.* **28** (1970) 599-608.
19. Mercier, C. and Feillet, P. *Cereal Chem.* **3** (1975) 283-296.
20. Varo, P., Laine, R. and Koivistoinen, P. *J. Assoc. Anal. Chem.* **66** (1983) 933-938.
21. Mercier, C. in 'Food Process Engineering' (P. Linko, Y. Mäklki, J. Olkku and J. Larinkari, eds) Applied Science Publishers Ltd, London (1980) vol. 1, pp 795-807.
22. Kim, J. C. and Rottier, W. *Cereal Foods World* **24** (1980) 62-66.
23. Owusu-Ansah, J., van der Voort, F. R. and Stanley, D. W. *Cereal Chem.* **60** (1983) 319-324.
24. Greenberg, C. J. *Studies on the fibre in human diets and its effect on the digestion and absorption of other nutrients. Dissertation.* University of Cambridge, England (1976).
25. Anderson, I. H., Levine, A. S., Levitt, M. D. *New Engl. J. Med* **15** (1981) 891-892.
26. Mörmann, J. E. and Mühlemann, H. R. *Caries Res.* **15** (1981) 166-175.
27. Frostwell, G. *Swed. Dent. J.* **65** (1972) 161-165.
28. Birkhed, D. and Fuchs, G. *Acta Odont. Scand.* **33** (1975) 59-66.
29. Imfeldt, T. *Schweiz Monatsschro Zahnheilkd.* **87** (1977) 437-464.
30. Birkhed, D. and Skude, G. *Scand. J. Dent. Res.* **86** (1978) 248-258.

31. Birkhed, D. and Edwardsson, S. in 'Health and Sugar Substitutes' Proceedings of ERGOB Conference, Geneva 1978, Karger, Basel (1978) pp 211-217.
32. Jenkins, D. J. A., Thorne, M. J., Camelon, K., Jenkins, A., Venketeshwer Rao, A., Taylor, R. H., Thompson, L. U., Kalmusky, J., Reichert, R. and Francis, T. *Am. J. Clin. Nutr.* **36** (1982) 1093-1101.
33. O'Dea, K., Snow, P. and Nestel, P. *Am. J. Clin. Nutr.* **34** (1981) 1191-1993.
34. Williams, C. A. and McDonald, I. *Proc. Nutr. Soc.* **41** (1982) 47A.
35. Skude, G. and Ihse, I. *Scand. J. Gastroenterol.* **11** (1976) 17-20.
36. Jenkins, D. J. A., Wolever, T. M. S., Leeds, A. R., Gassull, M. A., Haisman, P., Dilawari, J., Goff, D. V., Metz, G. L. and Alberti, K. G. M. *Br. Med. J.* **1** (1978) 1392-1394.
37. Holt, S., Heading, R. C., Carter, D. C., Prescott, L. F. and Tothill, P. *Lancet* **24** (1979) 636-639.
38. Garrow, J. S. in 'Obesity - Pathogenesis and Management' (J. T. Silverstone, ed.) Medical and Technical Publishing Co Ltd, Lancaster, England (1975), pp 1-27.

IV

EFFECTS OF PROCESSING ON STARCH AVAILABILITY

IN-VITRO AND IN-VIVO :

DRUM-DRYING OF WHEAT FLOUR

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SUMMARY

The effect of drum-drying on starch availability in white wheat flour was studied and compared with boiling and pressure cooking. The starch in two drum-dried products was less available to salivary amylase **in vitro** than in the corresponding boiled or pressure-cooked material. The rate of starch absorption in young rats, as reflected by the plasma glucose levels, was also slower with drum-dried products than with a boiled control. Similarly, the rise in plasma insulin was slower in drum-dried products. In addition to the conditions used during drum-drying, the method of preparation of the pre-cooked product affected starch availability, the rate of hydrolysis **in vitro** and, especially, the **in vivo** glucose response which was higher in products that were drum-dried and then boiled. The fermentability of drum-dried and boiled wheat flour in human dental plaque was similar, resulting in approximately the same pH drop **in situ**. A new type of amylose-lipid complex was formed during drum-drying. Under severe drying conditions, the dietary fibre content in wheat flour increased about 20% due to formation of enzyme resistant starch.

INTRODUCTION

Cooking and gelatinization of starch increases the susceptibility to enzymic degradation *in vitro*¹ as well as the availability for digestion and absorption in the small intestine². In addition, the physical structure of the product is important in relation to susceptibility to enzymic attack. According to Mc Neill et al³, the solubility of the protein matrix encapsulating the starch granules was even more important than the degree of gelatinization in processed sorghum grain. Furthermore, the particle size, i.e. the surface area/starch ratio, also affects the availability of cereal starches to enzymic digestion.

Only few studies exist on the effect of various industrial processes on starch availability *in vivo*. Slowly-absorbed carbohydrates are considered to be beneficial in the dietary management of diabetes. An increased availability may also favour development of dental caries because of the presence of amylase in the saliva⁵. In a recent study⁶, it was demonstrated that the starch in extrusion-cooked wheat products was more available to salivary amylase *in vitro*, and also more available for fermentation in dental plaque, compared to a corresponding boiled material. Moreover, the glycemic response to different wheat products administered to rats, was affected by the conditions used during extrusion, more severe conditions resulting in a higher response.

Another aspect on starch availability is in relation to dietary fibre analysis, as starch rendered enzyme-resistant during processing will be determined as dietary fibre⁷. The nutritional significance of this resistant starch fraction is not known.

Drum-drying is a commonly-used method in the food industry for pre-cooking of cereal based products such as gruels and porridge. Drying may cause retrogradation of starch⁸. According to Kayisu and Hood⁹, dehydration of cereal starches, including drum-drying, slightly reduced the susceptibility to α -amylase *in vitro*. The purpose of the present investigation was to study the effect of drum-drying on starch availability in white flour from wheat. Comparison was made with materials boiled at atmospheric pressure for 20 min and with pressure cooking (125°C, 20 min). The degree of gelatinization was evaluated by differential scanning calorimetry (DSC). Starch availability was studied both *in vitro* - using salivary amylase, and *in vivo* - by measuring the plasma glucose and insulin responses in young rats. The fermentability of drum-dried wheat flour in human dental plaque was evaluated by measuring the pH drop *in situ*. The effect of drum-drying on dietary fibre content was also studied.

EXPERIMENTAL

Commercially available white flour from wheat was obtained from Vaasa Mills Ltd, Finland. The quipment used for drum-drying was a pilot plant single drum from Goudsche Machinefabriek B.V. (Gouda, Holland). A suspension of raw wheat flour in water (250 g dry matter per litre) was administered directly on the drum with two applicator rolls. No pre-cooking was performed. The process temperature and the time for heat treatment was varied by changing the steam pressure inside the drum or the number of revolutions per minutes, respectively. The process conditions regarding steam pressure and drum speed were as follows: 4.9 bar, 13 rpm (product PI), 9.8 bar, 4 rpm (product PII). The two drum-dried products were sieved to pass a 1 mm screen. Product PI can be considered to be a realistic commercial product whereas product PII is slightly over-processed.

Starch analysis

The samples were gelatinized for 15 min in a boiling water bath before analysis. Starch content was estimated as glucose after incubation with amyloglucosidase (Boehringer Mannheim, W. Germany). The glucose formed was analyzed with a glucose oxidase reagent (Glox Novum, Kabi Diagnostica, Sweden). Starch content was calculated as enzymatically liberated glucose $\times 0.9^6$.

Differential Scanning Calorimetry (DSC)

DSC was performed on suspensions of raw, boiled and drum-dried wheat flour with a water-to-starch weight ratio of 10:1. The boiled control was prepared by heating a suspension of raw wheat flour for 20 min in a boiling water bath. The calibration of the DSC instrument (Perkin Elmer DSC-2) has been described elsewhere¹⁰. Approximately 10 mg of the test suspensions were transferred to weighed sample pans (Du Pont coated) which were sealed and reweighed. The samples were heated from 22°C to 127°C at a heating rate of 10°C/min, except on one occasion where heating was continued to 150°C. An empty sample pan was used as reference. The drum-dried flours were heated a second time after a rapid cooling in the instrument. The transition temperature (T) and the temperature interval (ΔT) were defined as illustrated in Fig 5, and the transition enthalpy (ΔH) was calculated from the peak area by cutting and weighing a recorder trace.

Determination of dietary fibre

The content of soluble and insoluble dietary fibre was analyzed gravimetrically after enzymic solubilization of protein and starch as described by Asp et al¹¹. Insoluble fibre was recovered by filtration. Soluble fibre was precipitated with 4 volumes of 96% ethanol and recovered by another filtration. The dietary fibre residues were corrected for remaining protein (assayed with the Kjeldahl procedure) and ash. All determinations were done in triplicate.

Starch hydrolysis *in vitro* using salivary amylase

A stock solution of salivary amylase was prepared by diluting saliva (1 ml) with 50% aqueous glycerol (40 ml) as described earlier⁶.

Amylase activity was measured at 37°C with soluble starch "nach Zulkowsky" (Merck, Darmstadt, W. Germany) as substrate.

The stock solution of salivary amylase was adjusted to contain 17 International Units (IU) of activity/ml (one IU catalyzes the liberation of 1 µmol maltose/min at 37°C). An amount of sample equivalent to 4 g starch (dry matter) was suspended in 0.05M K-Na-phosphate buffer, pH 6.9, containing 0.04% NaCl, and diluted to 100 ml.

A boiled wheat flour control was prepared by heating a suspension of raw wheat flour for 20 min in a boiling water bath with gentle stirring. Pressure cooking was performed in an autoclave at 125°C for 20 min. The drum-dried products were suspended as such, except in one case where the suspension was homogenized for 2 min with an Ultra Turrax homogenizer. The suspension was kept in an ice bath during homogenization to avoid heating of the sample. All suspensions were then diluted with 0.05M K-Na-phosphate buffer, pH 6.9 to a final starch concentration of 2 mg/ml. Substrate (10 ml), equivalent to 20 mg starch (dry matter), was incubated with 10 ml of a 1:20 dilution of the stock salivary amylase solution at 37°C. Samples (2 ml) of the incubation mixture were withdrawn at intervals during 2 h and mixed with dinitrosalicylic acid (DNS)-reagent¹². Maltose was used as standard, and the degree of starch hydrolysis (%) was calculated as $100 \times (\text{mg maltose equivalents/mg starch} \times 1.05)$. Soluble starch "nach Zulkowsky" was used as a control.

Experiments *in vivo* on young rats

Male rats (Sprague Dawley), weighing between 140 and 165 g, were given suspensions of the different wheat flour products by gastric intubation. The test suspensions were prepared by suspending the different drum-dried wheat flour products in 0.9% NaCl (100 ml) to a final starch concentration of 40 mg/ml, dry weight basis. Raw wheat flour, that had been gelatinized by heating for 20 min in boiling water, was used as a control. The suspensions were tempered in a water bath to 37°C and kept at this temperature during feeding. Ten animals were used for each product. Before the feeding experiments, the rats were fasted for 24 h but given free access to water. For each suspension, 2.5 ml, corresponding to 100 mg starch, were given through a stomach tube. Blood samples were taken from the retroorbital sinus¹³ for determination of plasma glucose and insulin. Both the gastric intubation and the blood sampling procedure were performed on conscious animals. Determination of plasma glucose was carried out with a glucose oxidase method¹⁴ and plasma insulin was analyzed with a radio-immuno assay¹⁵. The insulin values were expressed in international Units (U) as $\mu\text{U/ml}$ plasma ($25 \mu\text{U} \equiv 1 \text{ ng}$ insulin). After 2 h the animals were killed and a liver specimen (about 0.5 g) was taken for glycogen analysis¹⁶ from the ventral part of the left lateral lobe. Statistical evaluation was performed using Student's t-test.

Changes in pH of human dental plaque

Eighteen subjects were instructed not to clean their teeth for 2 days before the experiment and not to eat or drink on the morning of the examination. On the morning of the third day, the subjects rinsed their mouth with tap water to remove loose debris before rinsing for 30 s with the test suspension (10 ml). The following test suspensions were used, containing an equivalent amount of carbohydrate (5% w/v); glucose, soluble starch "nach Zulkowsky" boiled for 20 min, raw white flour, white flour boiled for 20 min and drum-dried white flour (PI). Samples of dental plaque were taken for pH determination immediately before (0 min plaque pH) and on five occasions (2, 5, 10, 20 and 30 min) after rinsing¹⁷. The different suspensions were administered to the subjects in a random order with an interval of 1 week between tests. Student's t-test for paired observations was used, comparing the pH values obtained with the test suspensions, or the decrease relative to the 0 min pH value, at different time intervals.

RESULTS

Starch availability *in vitro*

Starch content measured with amyloglucosidase was, on average, 74.4% (dry basis) in raw and drum-dried wheat flour products. No significant differences in starch content could be observed due to processing.

Drum-drying under conditions normally applied in the food industry for pre-cooking of cereals, as well as more severe process conditions rendered the starch in white flour less susceptible to hydrolysis by salivary α -amylase than boiling or pressure cooking (**Fig 1**). The shape of the hydrolysis curves differed considerably. Drum-drying gave a slow initial rate of hydrolysis with a levelling off at about 50% hydrolysis. After prolonged incubation of drum-dried flours, the degree of hydrolysis reached the level obtained with boiled flour. The conditions used during drum-drying also affected the starch availability *in vitro*. The flour processed under severe conditions (PII) showed a higher rate of amylolysis. Pressure cooking made the starch as susceptible as soluble starch. With these materials, the degree of hydrolysis levelled off at a point corresponding to about 80% hydrolysis. Raw wheat flour was hydrolyzed very slowly *in vitro*. The degree of hydrolysis after 2 h of incubation was less than 5%.

When suspensions of drum-dried white flour (PI) were boiled for 20 min or homogenized for 2 min, the availability to α -amylase increased slightly above the level obtained with boiled flour. The shape of the hydrolysis curves was similar to that obtained with boiled flour.

Starch absorption *in vivo* in young rats

Mild conditions during drum-drying (PI) resulted in a significantly lower plasma glucose response 15 and 30 min after intubation than did boiling (**Fig 2**). The wheat flour dried under more severe conditions (PII) also gave a lower response than boiled flour, although the difference in this case was less pronounced. Boiling of the drum-dried wheat flour product increased the peak glucose response, at 15 and 30 min, above the level obtained with flour after boiling alone.

In agreement with the plasma glucose pattern, the early insulin increment was lower with drum-dried than with boiled wheat flour (**Fig 3**). Furthermore, the peak insulin response following administration of the drum-dried materials was delayed compared to that with the boiled control. The wheat flour product that had been drum-dried and then boiled gave a similar early insulin response to that of the boiled sample, whereas the decrease in plasma insulin level with time seemed to be faster.

Liver glycogen levels 2 h after intubation were similar with boiled drum-dried products (**Table I**). However, the wheat flour product that was drum-dried (PI) and then boiled, resulted in a higher glycogen level than that seen with drum-dried (PI) or boiled flour, respectively.

Changes in pH of human dental plaque

The effect of rinsing the mouth with the various test suspensions is presented in **Fig 4**. Glucose resulted in the lowest plaque pH-values, followed by boiled soluble starch. Boiled or drum-dried (PI) wheat flour also induced a pH drop in the plaque, but the pH recovery was more rapid than with soluble starch or glucose. Raw wheat flour only had minor

effects on plaque pH. The statistical analysis is presented in **Table II**. No significant differences were found between drum-dried and boiled wheat flour, whereas boiled soluble starch resulted in slightly more pronounced pH decreases than did drum-dried flour (PI).

Differential Scanning Calorimetry

The DSC thermograms obtained with the different wheat flour suspensions are illustrated in **Fig 5**. With raw flour two endothermic peaks were observed. The first peak (50-75°C) corresponds to the gelatinization of starch¹⁸ whereas the second transition is caused by the disassociation of amylose-lipid complexes¹⁹. Only the second peak could be seen with boiled or drum-dried wheat flours. An endotherm due to retrograded amylose has been observed at about 150°C²⁰. No evidence of retrogradation was observed in the thermogram when sample PI was heated to such a high temperature.

The transition temperature, temperature interval and the transition enthalpy for the second endotherm are shown in **Table III**. As judged from the increase in ΔH , boiling and drum-drying increased the amount and/or the stability of the amylose-lipid complexes. The increase in ΔH was particularly pronounced in the case of drum-drying. Severe conditions (PII) resulted in a higher transition enthalpy than under milder conditions (PI). In addition, with drum-dried flours, the second endothermic peak had a very narrow temperature interval, about 6°C, compared with a ΔT of about 15°C or 21°C with raw or boiled flours, respectively. These results indicate that the amylose-lipid complexes formed during drying are more stable and of a higher order than those present in raw flour (i.e. heated in the DSC) or formed during boiling.

Reheating or homogenization of a suspension of drum-dried flour (PI) reduced the area of the second transition to the same level as that obtained after boiling. The shape of the peak was also similar to that seen with boiled flour, with a ΔT of about 22°C.

In **Table IV**, the percentage of complexed amylose in the different samples has been calculated from the ΔH value of the second transition. The transition enthalpy of pure amylose-lysolecithin complexes was used as a reference (ΔH 5.87 cal/g amylose)¹⁹, and the total amount of amylose in wheat starch was assumed to be 27%.²¹ Based on these figures, approximately 23% of the total amount of amylose in raw wheat flour, (i.e. heated in the DSC without any pretreatment) was complexed with lipids, compared with 45% after boiling. With drum-dried flours the percentage of complexed amylose appeared to be over 100%, indicating that ΔH for the complexes formed during drying were higher than the value for the amylose-lysolecithin complex used as reference. Reheating of drum-dried wheat flour or homogenization, reduced the amount of complexed amylose to 47%, i.e. to the same level as seen after boiling.

Dietary fibre content

The dietary fibre content in raw wheat flour and in wheat flour processed under mild conditions (PI) was similar, 3.7 and 3.8%, respectively (**Table V**). However, under more severe drying conditions (PII), there was an increase in analyzed dietary fibre content to 4.6%, i.e. about 20% increase. Only traces of starch could be detected in the fibre fractions by means of amyloglucosidase digestion. Thus, the increase in fibre content of about 1% (dry basis) under the severe conditions of processing seemed to be due to formation of a type of modified starch, resistant *in vitro* to both α -amylase and amyloglucosidase.

DISCUSSION

In this study, drum-dried wheat flour products were less available to α -amylase *in vitro* than autoclaved or boiled controls. However, after prolonged incubation of drum-dried wheat flour, the degree of hydrolysis reached the level obtained after boiling. The absence of the first endothermic peak in the DSC thermograms (50°C-75°C) with boiled and drum-dried flours indicate that the starch in these products was completely gelatinized. Thus, the differences observed in the availability to α -amylase cannot be explained by differences in the degree of gelatinization. However, from the thermograms it could be concluded that significant amounts of more stable amylose-lipid complexes were formed during drum-drying. Amylose-lipid complexes (lysolecithin) have been shown to be less available to α -amylase *in vitro* than free amylose, and as a consequence, digested and absorbed at a slower rate in the rat intestine²².

Boiling or homogenization of drum-dried wheat flour (PI) increased the availability to α -amylase. This can be interpreted as an improved hydration of dried wheat flour, or as an increase in substrate surface area. Suspensions of drum-dried flour were granular in appearance, whereas after boiling or homogenization this granular structure disappeared. The importance of physical form and substrate area for starch availability *in vitro* has been demonstrated recently with lentil starch²³.

Homogenization of boiled lentils produced a higher rate of starch digestion compared with boiled lentils mashed with a fork. Similar results were obtained by Snow and O'Dea¹ with wheat. After 30 min incubation *in vitro*, the degree of hydrolysis was 5%, 14% and 45% for rolled whole wheat, raw wholegrain wheat flour and cooked rolled wheat,

respectively. The rolled wheat was steamed before rolling, and thus was partly heat-treated. In spite of this, the raw flour was hydrolyzed faster, indicating the importance of surface area/starch ratio.

An additional explanation for an increased availability to enzymic hydrolysis after boiling or homogenization, could be that these processes affect the amylose-lipid complexes formed during drying. As judged from the decrease in ΔH , reheating or homogenization reduced the amount of amylose-lipid complexes and/or modified the complexes into a less stable structure. Amylose-lipid complexes disassociate at high temperature but reform on cooling^{19,24}. Our results show that, once disrupted by heat or otherwise, the characteristic complexes formed during drying do not reform. Homogenization was performed in an ice bath to avoid heating. Thus, it seems as if shear causes the amylose-lipid complexes to disassociate or transforms them into a less ordered structure. It has been suggested that complexes with a lower degree of order are more susceptible to enzymic degradation²².

In a previous study using the same *in vitro* technique, extrusion-cooked wheat flour products and autoclaved flour were equally available to salivary amylase⁶. That is, extrusion-cooking renders the starch more susceptible to degradation by α -amylase *in vitro* than boiling or drum-drying within the range of process conditions studied. When comparing extrusion-cooking and roll-cooking for gelatinization of cereal starch, differences in the physical properties of the products were found²⁵⁻²⁷. At a selected water absorption index suitable for preparation of gruels, the water solubility index was lower in drum-dried products. A low water solubility may reduce the susceptibility of starch to enzymic attack. Differences in availability of starch to enzyme attack *in vitro* have been reported also with other processes. In a study on wheat²⁸, the easily-hydrolyzed starch fraction represented 79%, 72%, 56% and 52%

of the total starch after extrusion, pelleting, popping and flaking, respectively, compared to only 8% in the raw material.

In agreement with other studies^{1,6,28} starch granules in raw wheat flour were relatively resistant to α -amylase **in vitro**. However, it is known that as with soluble starch, the over-all digestion of cereal starch **in vivo** is very efficient, almost 100%²⁹⁻³². It has been suggested that the discrepancy between the extent of digestion **in vitro** and **in vivo** of raw granules is due to some yet unknown modification taking place in the digestive tract³³. In addition, it was recently demonstrated that significant amounts of starch in white wheat bread were passed undigested to the colon in human subjects³⁴. Only very few data are available on the rate of starch absorption **in vivo** from raw (as compared with gelatinized) food items. However, Collings et al² demonstrated that the serum glucose and insulin responses in healthy subjects were significantly higher following ingestion of cooked, compared with raw starch. It has been suggested also that raw food has some potential in the dietary management of diabetes³⁵.

In this study, the **in vivo** availability of starch in rats was in good agreement with results obtained **in vitro** with boiled and drum-dried wheat flour (PI). Thus, the difference in the rate of digestion and absorption can be explained by assuming that digestion was rate limiting. A similar tendency was seen also with the flour processed under more severe conditions (PII). However, rats given this product had a significantly lower fasting plasma-glucose value, making interpretation more difficult. Administration of boiled drum-dried wheat flour (PI), resulted in a significantly higher plasma glucose response compared to that with boiled flour. This is in agreement with the **in vitro** assay, although the difference in availability to salivary amylase **in vitro** was small.

The cariogenic properties were similar with drum-dried (PI) and boiled wheat flour suspensions, as judged from the pH measurement in dental plaque. Thus, in spite of the difference in the rate of starch digestion and absorption between these materials, the starch was equally available to fermentation in the plaque. However, in agreement with the *in vitro* assay, the raw wheat flour product had only minor effects on plaque pH, whereas soluble pure starch produced significantly lower pH-values at several time intervals. In contrast, starch in extruded wheat flour was equally available for fermentation as soluble starch⁶. A more extensive discussion regarding the plaque-pH method and the cariological considerations of different starch products was presented in the previous study⁶.

The increase in dietary fibre content of about 20% after drum-drying (PII) is comparable to that reported during extrusion-cooking at severe conditions³⁶. In contrast to extruded materials, no redistribution between insoluble and soluble dietary fibre was observed. The increase in dietary fibre at severe drum-drying conditions, was probably due to formation of starch resistant to the α -amylases (Termamyl and pancreatin) used in the dietary fibre assay. It is not likely that amylose-lipid complexes contribute to the dietary fibre value, as these complexes are solubilized at the high temperature used during incubation with Termamyl (100°C)²². The starch residue formed during drying was also resistant to amyloglucosidase. In extruded wheat flour, on the other hand, remaining starch could be removed from the fibre residues with a subsequent incubation with amyloglucosidase³⁶. Formation during bread baking of "resistant starch", available to amyloglucosidase only after solubilization with KOH has been reported⁷.

The differences in starch availability after processing of wheat flour in this study may reflect differences in physical form, suspensions of drum-dried flour being less homogenous. However, the conditions during drum-drying led to formation of more stable amylose-lipid complexes. In drum-dried flours all the amylose was involved, corresponding to approximately 27% of the total amount of starch. To account for the lower availability of starch in these products, it seems probable that the insoluble amylose-lipid complexes formed on the starch granule surface provide a barrier to enzymic attack.

In addition to the process conditions during pre-cooking, the method used when preparing the pre-cooked product before consumption seems to be important. That is, whether the product is suspended in water and consumed as such or boiled before consumption. In this study, the *in vitro* results were in good agreement with those obtained *in vivo*. However, with extruded and boiled wheat flour products, the agreement was not so consistent⁶. In view of this, the glycemic response to starch after various food processes, as well as the effect on plaque-pH, are important fields for further research. Also important is the development of a reliable *in vitro* system to be used for a possible prediction of the glycemic response *in vivo*.

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REFERENCES

1. Snow, P., and O' Dea, K. **Am. J. Clin. Nutr.** **34** (1981) 2721-2727
2. Collings, P., Williams, C. and Mc Donald, J. **Br. Med. J.** **282** (1981) 1032
3. Mc Neill, J.W., Potter, G.D., Riggs, J.K. and Rooney, L.W. **J. Animal Sci.** **40** (1975) 335-341
4. O' Dea, K., Nestel, P.J. and Antonoff, L. **Am. J. Clin. Nutr.** **33** (1980) 760-765
5. Mörmann, J. and Mühlemann, H.R. **Caries Res.** **15** (1981) 166-175
6. Björck, I., Asp, N.-G., Birkhed, D. and Lundquist, I. **J. Cereal Sci.** (1984 in press)
7. Johansson, C.-G., Siljeström, M. and Asp, N.-G. **Z. Lebensm. Unters. Forsch.** (1984 submitted)
8. Hellendoorn, E.W., van der Top, M., and van der Weide, J.E.M. **J.Sci.Fd.Agric.** **21** (1970) 71-75.
9. Kayiso, K. and Hood, L.F. **J. Food Sci.** **44** (1979) 1728-1731
10. Eliasson, A.-C. **J. Cereal Sci.** **1** (1983) 199-205
11. Asp, N.-G. Johansson, C.-G., Hallmer, H. and Siljeström, M. **J. Agric. Food Chem.** **31** (1983) 476-482
12. Hostettler, F., Borel, E. and Duel, H. **Helv. Chim. Acta** **34** (1951) 2132-2139
13. Rerup, C., and Lundquist, I. **Acta Endocrinol.** (Kbh) **52** (1966) 357-367
14. Bruss, M.L. and Black, A.L. **Analyt. Biochem.** **84** (1978) 309-312
15. Heding, L.G. in " Labelled proteins in tracer studies " (L. Donato et al eds.) Euratom, Brussels (1966) pp 345-350
16. Rerup, C. and Lundquist, I. **Acta Pharmacol. Toxicol.** **25** (1967) 41-53
17. Frostell, G. **Acta Odont. Scand.** **28** (1970) 599-608

18. Stevens, D.J. and Elton, G.A.H. **Stärke** **23** (1971) 8-11.
19. Kugimiya, M. and Donovan, J.W. **J. Food Sci.** **46** (1981) 765-777.
20. Stute, R. and Konieczny-Janda, G. **Stärke** **35** (1983) 340-347.
21. Morrison, W.R. and Laignelet, B. **J. Cereal Sci.** **1** (1983) 9-20.
22. Holm, J., Björck, I., Ostrowska, S., Eliasson, A.-C., Asp, N.-G., Larsson, K. and Lundquist, I. **Stärke** **35** (1983) 294-297.
23. Wong, S. and O'Dea, K. **Am. J. Clin. Nutr.** **37** (1983) 66-70.
24. Eliasson, A.-C. Physical properties of starch in concentrated systems such as dough and bread. Dissertation 1983. Dept. of Food Techn., University of Lund, Sweden
25. Anderson, R.A., Conway, H.F., Pfeifer, V.F. and Griffin, E.L. **Cereal Sci. Today** **14** (1969) 4-7, 11-12
26. Anderson, R.A., Conway, H.F., Pfeifer, V.F. and Griffin, E.L. **Cereal Sci. Today** **14** (1969) 372-375, 381-382
27. Anderson, R.A., Conway, H.F. and Peplinski, A.J. **Stärke** **22** (1970) 130-134
28. Delort-Laval, J. and Mercier, C. **Ann. Zootech.** **25** (1976) 3-12
29. Booher, L.E., Behan, J. and Mc Means, E. **J. Nutr.** **45** (1951) 75-95
30. Fleming, S.E. and Vose, J.R. **J. Nutr.** **109** (1979) 2067-2075
31. Wolf, M.J., Khoo, U. and Inglett, G.E. **Stärke** **29** (1977) 401-405
32. Isaksson, G., Ahre'n, B. Ihse, I. and Lundquist, I. **Acta Endocrinol.** **103** (1983) 376-384
33. Marshall, J.J. and Whelan, W.J. **Congr. Fed. Asian Oceanian** (1979) 125-130
34. Anderson, I.H., Levine, A.S. and Levitt, M.D. **New Eng. J. Med.** **15** (1981) 891-892
35. Douglass, J.M. **Ann. Int. Med.** **82** (1975) 61-63
36. Björck, I., Nyman, M. and Asp, N.-G. **Cereal Chem.** (1984 in press)

Table 1. Liver glycogen content in rats 2h after intubation with suspensions of boiled and drum-dried wheat flour

Wheat flour product	Liver glycogen content ^a (mg/ g wet weight)
Boiled	5.3 ± 1.1
Drum-dried (PI)	4.5 ± 1.0
+ boiled	9.4 ± 1.5 b
Drum-dried (PII)	5.8 ± 0.6

^a Mean values ± sem

^b Significantly different from other samples, $p < 0.05$. (Student's t-test)

Table II. Significant differences^a in plaque pH at different time intervals after rinsing with the following test suspensions (5% w/v carbohydrate) : Raw wheat flour, boiled wheat flour, drum-dried wheat flour (PI), boiled soluble starch and glucose

pH	Time after mouth rinsing (min)										
	0	2	$\Delta(0-2)$	5	$\Delta(0-5)$	10	$\Delta(0-10)$	20	$\Delta(0-20)$	30	$\Delta(0-30)$
Drum-dried flour (PI) - raw flour	-	***	***	***	***	**	***	-	-	*	*
Drum-dried flour (PI) - boiled flour	-	-	-	-	-	-	-	-	-	-	-
Drum-dried flour (PI) - soluble starch	-	-	**	-	*	-	*	-	-	-	*
Drum-dried flour (PI) - glucose	-	-	*	**	**	**	**	**	**	**	**

^a Student's paired t-test : (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$, - not significant
Calculations performed on 18 subjects

Table III. Transition temperature (T), temperature interval (ΔT) and transition enthalpy (ΔH) for amylose-lipid complexes in raw and processed wheat flour. Mean value and standard deviation of three DSC scans.

Wheat flour product	T ($^{\circ}\text{C}$)	ΔT ($^{\circ}\text{C}$)	ΔH (cal/g dry matter)
raw	91.9 ± 0.2	14.7 ± 1.6	0.27 ± 0.07
boiled	100.9 ± 0.7	21.4 ± 1.9	0.54 ± 0.09
drum-dried (PI)	97.1 ± 0.1	6.0 ± 0.1	1.20 ± 0.17
reheated	93.6 ± 2.3	24.0 ± 0.9	0.56 ± 0.10
homogenized	96.1 ± 0.4	19.0 ± 5.0	0.55 ± 0.17
drum-dried (PII)	95.6 ± 0.7	5.9 ± 1.8	1.67 ± 0.10
reheated	94.9	23.1	0.55

Table IV. Calculated percentage of complexed amylose in raw and processed wheat flour^a

	% of total amount of amylose ^b	
	raw ^c	
raw ^c	23	
boiled	45	
drum-dried (PI)	102	
reheated	47	
homogenized	47	
drum-dried (PII)	142	
reheated	47	

^a ΔH of a purified amylose-lysolecithin complex was used as reference material¹⁹.

^b calculations based on an amylose content in wheat starch of 27%²¹ by weight.

^c control heated in the DSC without any pre-treatment

Table V. Dietary fibre content of raw and drum-dried wheat flour

Product	Dietary fibre (% dry basis)		
	Insoluble	Soluble	Total
raw wheat flour	2.1 $\pm$ 0	1.5 $\pm$ 0.1	3.7 $\pm$ 0.2
drum-dried (PI)	2.0 $\pm$ 0.1	1.7 $\pm$ 0.3	3.8 $\pm$ 0.2
drum-dried (PII)	2.6 $\pm$ 0.1 ^a	2.0 $\pm$ 0.4	4.6 $\pm$ 0.3 ^a

a significantly different from raw material $P < 0.01$ (Student's t-test)

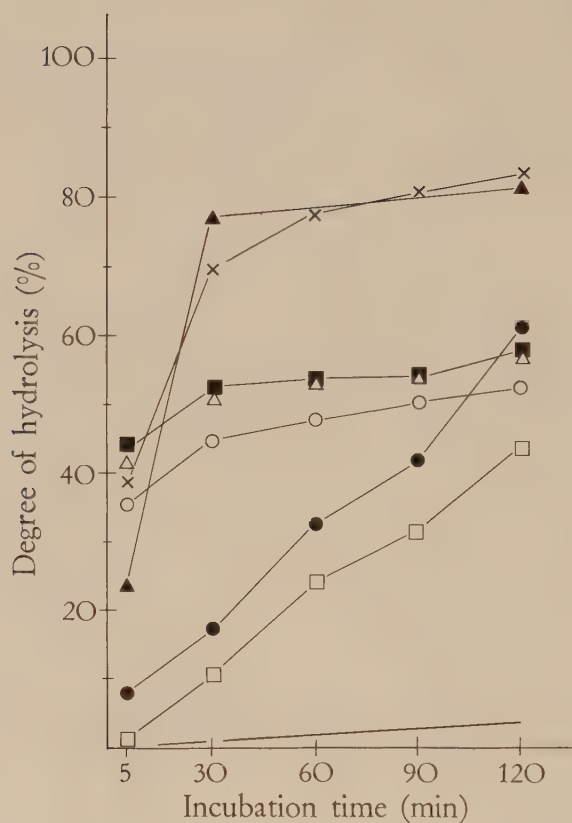


Fig 1. Starch hydrolysis with salivary amylase in vitro.

The same starch concentration was used with all samples.

The degree of hydrolysis is expressed as maltose equivalents.

Wheat flour

raw (—)^a

boiled (○)

autoclaved (▲)

drum-dried;

mild conditions PI (□)

— then homogenized (△)

— then boiled (■)

severe conditions PII (●)

Soluble starch "nach Zulkowsky" (×)

^a due to low levels of hydrolysis with raw wheat flour,
no experimental points are marked

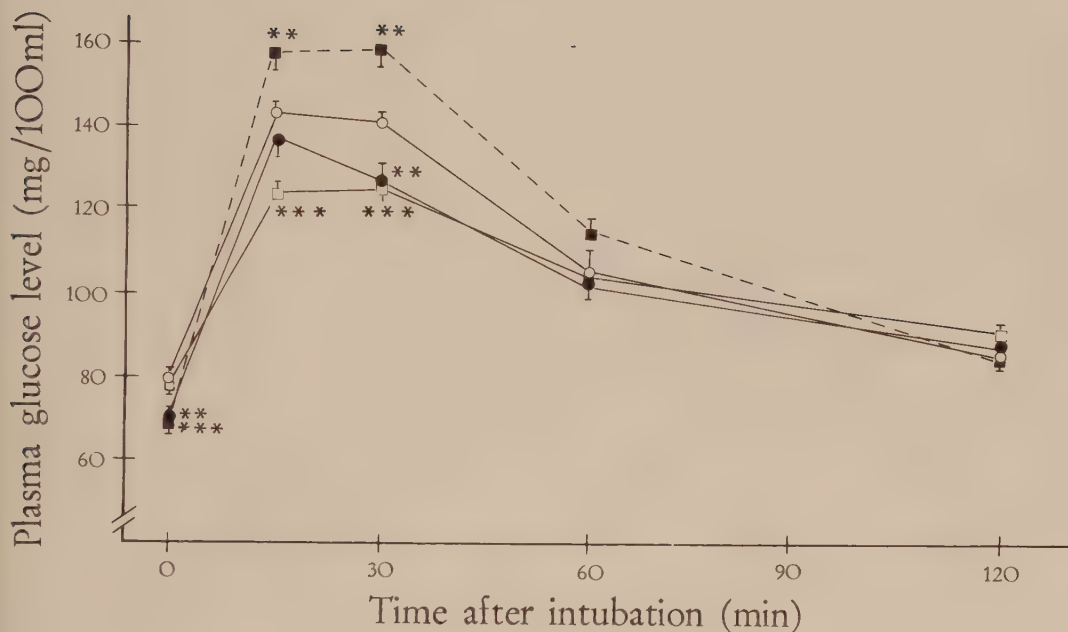


Fig 2. Plasma glucose levels in rats following a gastric load containing 100 mg starch as a 4% w/v suspension in 0.9% NaCl.

Wheat flour

boiled (○)

drum-dried PI (□)

+ boiled (■)

drum-dried PII (●)

10 rats in each group. Mean values $\pm$ s.e.m.

Statistical evaluation: Student's t-test,

** $p < 0.01$ and *** $p < 0.001$

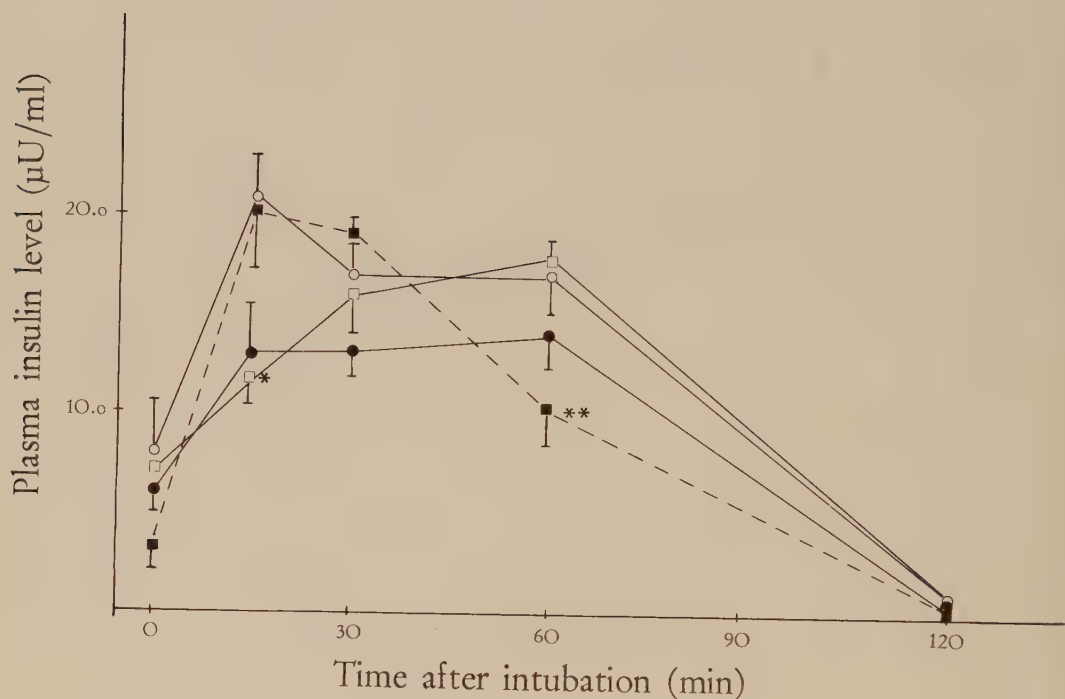


Fig 3. Plasma insulin levels^a in rats following a gastric load containing 100 mg starch as a 4% w/v suspension in 0.9% NaCl.

Wheat flour

boiled (○)

drum-dried PI (□)

+ boiled (■)

drum-dried PII (●)

10 rats in each group. Mean values $\pm$ s.e.m.

Statistical evaluation: Student's t-test, * $p < 0.05$ and ** $p < 0.01$

a 25 μ U equals 1 ng insulin

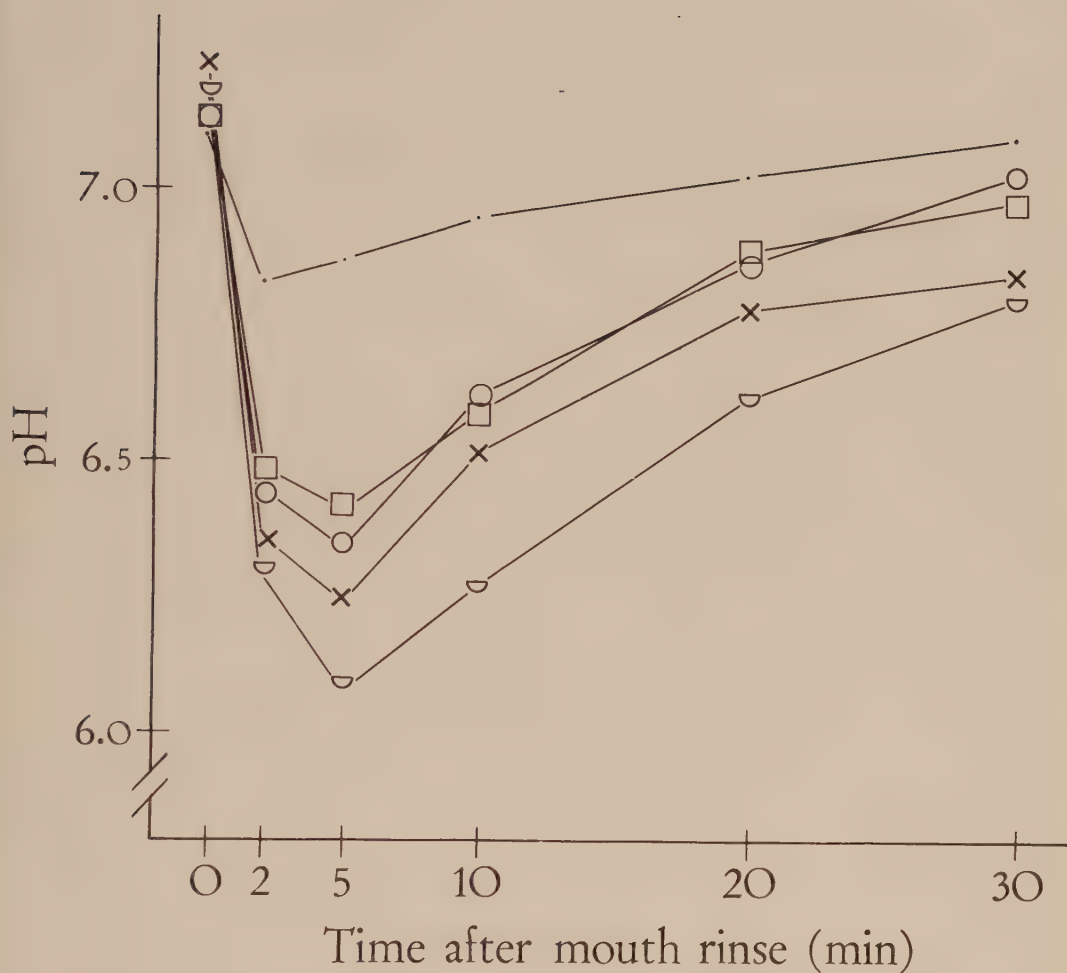


Fig 4. Changes in pH of human superficial dental plaque in situ after a 30 s mouth rinse with 10 ml of the following test suspensions (5% w/v carbohydrate) : glucose (U), boiled soluble starch (X), raw white flour (•), boiled white flour (O) and drum-dried white flour PI (□). Mean values of 18 subjects.

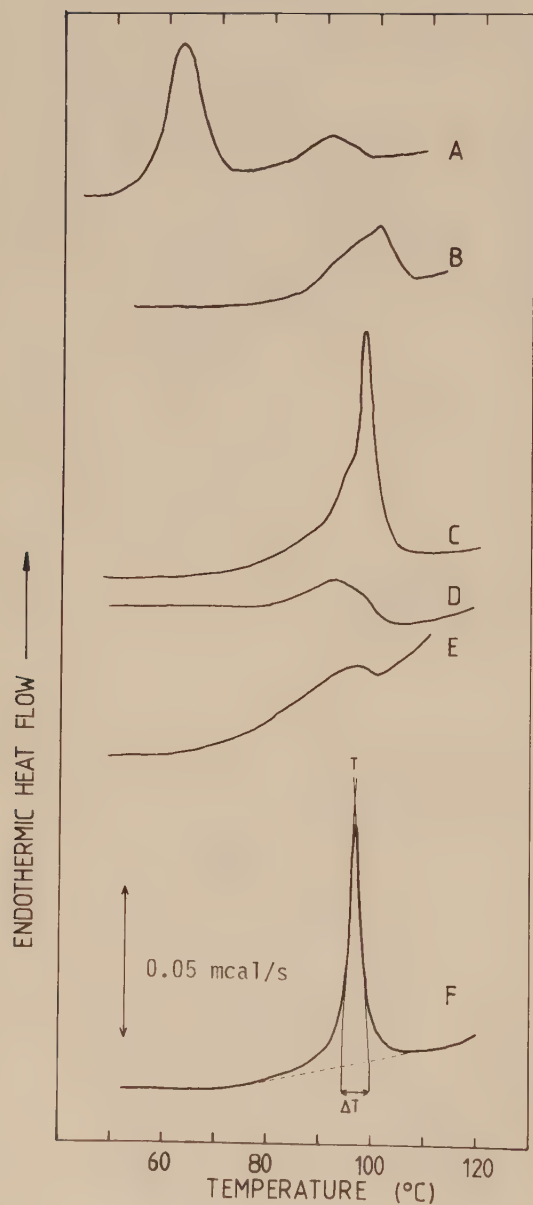


Fig 5.

Thermograms of white wheat flours.
Weights of flours (mg)

A=raw flour (1.35)

B=boiled flour (2.70)

C=drum-dried flour PI (1.88)

D=C heated a second time in
the DSC

E=drum-dried flour PI,
homogenized (1.90)

F=drum-dried flour PII (1.20)

HORMONAL AND METABOLIC RESPONSES TO BREAKFAST MEALS
IN NIDDM : COMPARISON OF WHITE AND WHOLE-GRAIN WHEAT
BREAD AND CORRESPONDING EXTRUDED PRODUCTS

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submitted for publication

SUMMARY

The post-prandial glucose and hormonal responses were followed in nine non-insulin-dependent diabetics after four randomized breakfast meals containing mainly wheat products. The effect of conventionally baked breads was compared with extruded crispbread-like products prepared from white or whole-grain wheat flour, respectively. The extruded whole-grain product gave significantly larger areas under the glucose and insulin curves than the corresponding baked bread, and resulted in higher C-peptide, gastric inhibitory polypeptide, and glucagon concentrations at certain time intervals. There were no significant differences between white and whole-grain baked bread although a tendency to higher blood glucose response after white bread was observed. We conclude that extrusion cooking increases the post-prandial glucose and hormonal responses in non-insulin-dependent diabetes mellitus (NIDDM). The lack of difference in response to white wheat and whole-grain wheat products indicates that dietary fibre in wheat is not very efficient in modulating these parameters.

Key words:

Diabetes mellitus, dietary fibre, wheat, extrusion cooking, blood glucose, immunoreactive insulin, C-peptide, gastric inhibitory polypeptide, glucagon, triglycerides, glycerol.

INTRODUCTION

It is now well established that great differences exist in the rate of digestion and absorption of starch from different food items (1-4). However, less is known about the effect on starch availability of different food processes, e.g. the degree of refining of flour or the method used for heat treatment. Extrusion cooking is a continuous process for cooking and forming of cereals which is increasingly used for the production of crispbread-like products, breakfast cereals and gruels (5,6). In a recent study (7), the conditions during extrusion cooking of wheat products were shown to affect the post-prandial glucose and insulin responses in rats.

The present investigation was performed to study the short-term metabolic effects in man of dietary fibre content in wheat bread and of extrusion cooking versus conventional baking. The post-prandial glucose and hormonal responses were determined in nine non-insulin-dependent diabetics given baked or extruded bread products, from white and whole-grain wheat flour respectively, as the main source of carbohydrate in a breakfast meal.

MATERIALS AND METHODS

Test meals

Commercially available wheat flours were used, white wheat flour (75 % extraction rate) and whole-grain wheat flour (Kungsörnen AB, Sweden). From these raw materials four different bread products were prepared either by conventional oven baking or by extrusion cooking. Details about the baking procedure are given in Table 1. Extrusion cooking takes place at elevated temperature and pressure and at a lower water content. In the extruder, the ingredients are subjected to intense mechanical treatment by rotating screws. The combined effect of heat, pressure and shear transform the material into a dough-like mass which is pressed through a die (5). When passing the die, the product receives a shape and a porous structure resembling that of a crispbread. The conditions used during extrusion cooking are shown in Table 2.

The patients were given 104.5 g whole-grain bread (WgB), 83 g white bread (WB), 61 g extruded whole-grain product (WgE) or 48.5 g extruded white wheat product (WE), corresponding to an equivalent amount of starch. In addition to these products, the meals contained low fat milk (0.5 % fat, 100 g), margarine (80 % fat, 10 g), soft cheese (10 % fat, 30 or 35 g), tea or coffee (150 g) and water (200 g). The larger amount of cheese was given with the two meals based on white wheat flour products to compensate for the somewhat higher content of protein and fat in the whole-grain products (Table 3).

Patients

Nine patients with established non-insulin-dependent diabetes mellitus (NIDDM) were included in the study. None had any antidiabetic treatment except conventional dietary advice. All patients gave informed consent and the study was approved to by the Ethical committee of Lund University.

The patients, 6 men and 3 women, were regular attenders at the outpatient clinic in Dalby, Sweden. They ranged in age from 43 to 83 years (63 ± 11 years, mean $\pm$ SD). In all cases the NIDDM was diagnosed at least 12 months before the start of the study. Clinical data for the individual patients are shown in Table 4. The mean fasting blood glucose concentration was 8.9 ± 2 mmol/l (mean $\pm$ SD), the total glycosylated haemoglobin (HB A1) amounted to 10.3 ± 1.6 % and the body mass index was 29.0 ± 6.1 kg/m².

Each subject was given the four test meals randomized at intervals of at least one week. The studies started at 8 a.m., after an overnight fast, and the time to complete the meal was 19 ± 8 min. Samples of venous blood were drawn at 10-30 min intervals until 3 h after the meal.

Analysis of test meals

Starch content was analyzed enzymatically using amyloglucosidase (7) after a modified gelatinization step containing a thermostable α -amylase (8). The total dietary fibre content was determined with an enzymatic gravimetric method (8). Nitrogen was

measured with the Kjeldahl procedure, and protein content was calculated using the conversion factor for cereals, 5.7. The fat content was analyzed gravimetrically by extraction with ether/petrolether after acid hydrolysis (9).

Analysis of blood parameters

Blood glucose was determined with the hexokinase method (10). The plasma concentrations of immunoreactive insulin (IRI) and connecting peptide (C-peptide) were measured according to Heding (11,12). Gastric inhibitory polypeptide (GIP) was determined as described by Kuzio et al (13) and glucagon was measured according to Faloona and Unger (14). HbA1 was determined as described previously (15,16). Plasma triglycerides and glycerol were determined enzymatically with reagents from Boehringer-Ingelheim and WACO, respectively (17,18).

Statistical analysis

Significance of differences were evaluated using Wilcoxon signed ranking test.

RESULTS

Test meals

The contents of starch, dietary fibre, protein and fat in the bread products are given in Table 5 and the composition of the breakfasts is shown in Table 3. The amounts of starch, protein, fat and energy were similar in all four test meals. The total dietary fibre content of the meals ranged from 1.6-8.9 g.

The patients consumed the bread without any problems, but for some patients the extruded products were difficult to eat within the same period of time, due to their larger volume. The durations of the meals were (mean $\pm$ SEM) 17.5 $\pm$ 1.6 (WgB), 24.3 $\pm$ 3.4 (WgE), 13.1 $\pm$ 1.3 (WB), and 21.6 $\pm$ 3.1 (WE) min. The difference between WB and WE was significant, ($p < 0.01$).

Hormonal and metabolic responses

The areas under the curves for blood glucose and plasma-IRI were higher ($p < 0.05$) after the breakfast containing WgE as compared to WgB (Table 6). The glucose values were not significantly different at any specific time, but the plasma-IRI and C-peptide levels were higher ($p < 0.01$) after WgE at 150'. Further, GIP concentrations were higher at 30' ($p < 0.05$) and glucagon levels were higher at 45' ($p < 0.05$) and 60' ($p < 0.01$).

When comparing the test meals containing WgB or WB no significant difference was found in any variable at any time point. The areas under the curves during the 0-180 min. period were also similar (Table 6, Fig. 2).

The responses in blood glucose and GIP were higher ($p < 0.05$) after WE at 135' and 120' respectively, compared with the corresponding baked product, WB (Table 6). However, plasma-IRI and plasma-C-peptide did not differ, and the areas under the curves were similar for all variables studied.

The triglyceride and glycerol responses were similar after all the breakfasts (Fig. 1, Table 6).

DISCUSSION

Bread constitutes an important source of carbohydrate in the human diet. Earlier studies on the effect of flour extraction rate, that is the dietary fibre content, on the glycaemic response to wheat bread are contradictory. In some studies whole-grain wheat bread has been reported to cause a lower post-prandial glucose response in healthy subjects than white bread (19,20). On the other hand, no effect of the naturally occurring fibre in whole-grain wheat bread was observed by Jenkins et al (4,21,22). However, addition of wheat bran to the diet during a longer period has been shown to improve glucose tolerance in human subjects (23).

In the present study, the higher dietary fibre in whole-grain bread (WgB) did not influence the post-prandial levels of insulin, C-peptide, GIP, glucagon, triglycerides or glycerol in NIDDM compared with the corresponding white bread (WB). However, the glucose response area (0-180 min) was about 20 % larger after WB although the difference did not reach statistical significance (Fig. 2).

The conditions during baking have been shown to have an impact on the availability of starch in bread. According to Snow and O'Dea (24), the starch in commercially baked bread was more susceptible to enzymatic degradation *in vitro* than starch in the corresponding home-made bread. Further, an increase in baking time significantly increased the glycaemic response to bread in healthy subjects (25). In our study, the same baking time and oven temperature were used during baking of white and whole-grain bread (Table 1).

Several extruded bread-like products are now commercially available. In a recent study (7), the process conditions during extrusion cooking of wheat flour and whole-grain wheat flour affected the post-prandial glucose levels in rats. Products extruded under severe conditions produced significantly higher glucose responses than the corresponding boiled controls.

The extrusion conditions used in the present study must be considered very mild. Still, intake of an extruded whole-grain product in a composite breakfast meal resulted in somewhat higher glucose and hormonal responses than a breakfast containing the corresponding baked bread. A possible explanation could be differences in the degree of gelatinization. Gelatinization of starch granules increases the susceptibility to enzymic digestion and absorption (24, 26). In spite of the low water content during extrusion cooking, complete gelatinization is usually obtained even at moderate process temperatures (27). However, considerable amounts of starch granules remain intact during conventional baking, particularly in the crust (28). Consequently, a reduced post-prandial response to wheat bread was observed with an increasing crust/crumb ratio in healthy subjects (29).

No differences were found between the two white bread products, WB and WE, when calculating the areas under the glucose or insulin curves. However, the blood glucose response and GIP levels were higher after the meal containing the extruded product at 135' and 120', respectively ($p < 0.05$). The difference between WB and WE might have been more evident if the patients had been able to eat these products within the same period of time. The slower ingestion of WE ($p < 0.01$) compared with WB may have concealed that the starch in the extruded product was more easily available.

In a previous study (30) it was demonstrated that a breakfast containing whole-grain bread and whole apples produced a significantly lower post-prandial glucose elevation in NIDDM than the corresponding breakfast with white bread and apple juice. The fibre effect in that study may have emanated from the apple or from the bread which in addition to wheat also contained rye, oat and barley. These cereals contain more soluble fibre than wheat (31). Soluble viscous fibres, like pectin and guar gum, reduce the post-prandial glycaemia both in healthy and in diabetic subjects (32,33).

In conclusion, the naturally occurring dietary fibre in whole-grain wheat bread did not significantly affect the post-prandial glucose and hormonal responses in our short-term study. Extrusion cooking of whole-grain wheat flour produced significantly higher blood glucose and insulin responses than did baking. Although the differences were small, we conclude that products from conventional baking are to be preferred to extruded products in non-insulin-dependent diabetes mellitus.

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References

- 1 Crapo PA, Reaven G, Olefsky J (1977) Postprandial plasma-glucose and -insulin responses to different complex carbohydrates. *Diabetes* 26:1178-1183
- 2 Reaven GM (1979) Effects of differences in amount and kind of dietary carbohydrate on plasma glucose and insulin responses in man. *Am J Clin Nutr* 32:2568-2578
- 3 Jenkins DJA, Wolever TMS, Taylor RH, Ghafari H, Jenkins AL, Barker H, Jenkins MJA (1980) Rate of digestion of foods and post-prandial glycaemia in normal and diabetic subjects. *Brit Med J* 5:14-17
- 4 Jenkins DJA, Wolever TMS, Taylor RH, Barker H, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL, Goff DV (1981) Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr* 34:362-366
- 5 Harper JM (1981) Extrusion of foods. Vol I. CRC press, Boca Raton, Florida
- 6 Björck I, Asp N-G (1983) The effects of extrusion-cooking on nutritional value - A literature review. *J Food Eng* 2:281-303
- 7 Björck I, Asp N-G, Birkhed D, Lundquist I. Effects of processing on starch availability in-vitro and in-vivo - Extrusion cooking of wheat flours and starch. *J Cereal Science* (in press)
- 8 Asp N-G, Johansson C-G, Hallmer H, Siljeström M (1983) Rapid enzymatic assay of insoluble and soluble dietary fibre. *J Agric Food Chem* 31:476-482
- 9 Association of Official Analytical Chemists (1980) Determination of fat. Official methods of analysis. In: Horwitz W (ed) The Society, Washington DC, 14.019 p 213

- 10 Coburn HJ, Carrol JJ (1973) Improved manual and automated colorimetric determination of serum glucose, with use of hexokinase and glucose-6-phosphate dehydrogenase. *Clin Chem* 19:127
- 11 Heding L (1966) A simplified insulin radioimmunoassay method. In: Donato L, Milhaud G, Sirchis J (ed) *Euratom*, Brussels, p 345-350
- 12 Heding L (1975) Radioimmunological determination of human C-peptide in serum. *Diabetologia* 11:541-548
- 13 Kuzio M, Drybugh JR, Malloy KM, Brow JC (1974) Radioimmuno assay for gastric inhibitory polypeptide. *Gastroenterology* 66:357-364
- 14 Faloona GR, Unger R H (1974) Glucagon. In: Jaffe BM, Behrman HR (ed) *Methods of Hormone Radioimmunoassay*. Academic Press, New York p. 317-330.
- 15 Allen DW, Schroeder WA, Balog J (1958) Observations on the chromatographic heterogeneity of normal adult and fetal human hemoglobin: a study of the effects of crystallization and chromatography on the heterogeneity and isoleucine content. *J Am Chem Soc* 80:1628-1634
- 16 Nathan DM, Avezzano ES, Palmer JL (1981) A rapid chemical means for removing labile glyco-hemoglobin. *Diabetes* 30:700-701
- 17 Wahlefeld AW (1974) Triglyceride determination after enzymatic hydrolysis. In: Bergmeyer HN (ed) *Methods of Enzymatic Analysis*. Verlag Chemie Weinheim and Academic Press, New York, p 1831
- 18 Hanrek S, Tibbling G (1966) An enzymatic fluorimetric micromethod for the determination of glycerol. *Clin Chem Acta* 4:395-400

- 19 Greenberg CJ (1976) Studies on the fibre in human diets and its effect on digestion and absorption of other nutrients. Dissertation. University of Cambridge, England
- 20 Thomas B, Elchazly M (1976) Funktionelle Wirkungen und Veränderungen der Ballaststoffe des Weizens während des Verdauungsablaufes. Qual plant - Pl Fds, Hum Nutr 1(3):211-226
- 21 Jenkins DJA, Wolever TMS, Taylor RH, Barker HM, Fielden H, Gassull MA (1981) Lack of effect of refining on the glycemic response to cereals. Diabetes Care 4(5):509-513
- 22 Jenkins DJA, Wolever TMS, Jenkins AL, Lee R, Wong GS, Josse R (1983) Glycemic response to wheat products: Reduced response to pasta but no effect of fiber. Diabetes Care 6(2):155-159
- 23 Brodribb AJM, Humphreys DM (1976) Diverticular disease: three studies. Part III: Metabolic effects of bran. Br Med J 1:428-430
- 24 Snow P, O'Dea K (1981) Factors affecting the rate of hydrolysis of starch in food. Am J Clin Nutr 34:2721-2727
- 25 Lüder W, Aust L, Vetter K (1969) Zum Verlauf der alimentären Hyperglykämie nach Brotverzehr. Die Nahrung 13(3):191-198
- 26 Collings P, Williams C, MacDonald I (1981) Effects of cooking on serum glucose and insulin responses to starch. Br Med J 282:1032
- 27 Rabe E, Seiler K, Gödecke V (1980) Analytische Bestimmungen der Stärkeverkleisterungen von Extrudaten. Zucker Süßwaren Wirtsch 33(5):158-162
- 28 Varriano-Marston E, Huang VKEG, Ponte J (1980) Comparison of methods to determine starch gelatinization in bakery foods. Cereal Chemistry 57(4):242-248

- 29 von Glatzel H, Rettenmaier G (1962) Ernährungsphysiologische Brotvergleiche IV. Der Verlauf der alimentären Hyperglykämie. *Nutr Dieta* 4:289-296
- 30 Hagander B, Scherstén B, Asp N-G, Sartor G, Agardh C-D, Schrezenmeir J, Kasper H, Ahrén B, Lundquist I (1984) Effect of dietary fibre on blood glucose, plasma immunoreactive insulin, C-peptide and GIP responses in non insulin dependent (type 2) diabetics and controls, *Acta Med Scand* (in press)
- 31 Nyman M, Siljeström M, Pedersen B, Bach-Knudsen K-E, Asp N-G (1984). Dietary fibre content and composition in six cereals at different extraction rates. *Cereal Chemistry* 61:14-19.
- 32 Jenkins DJA, Goff DV, Leeds AR, Alberti KGMM, Wolewer TMS, Gassull MA, Hockaday TDR (1976) Unabsorbable carbohydrates and diabetes: decreased postprandial hyperglycaemia. *The Lancet* 24:172-176
- 33 Jenkins DJA, Wolewer TMS, Leeds AR, Gassull MA, Haisman P, Dilawari J, Goff DV, Metz GL, Alberti KGMM (1978) Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. *Br Med J* 1:1392-1394

Table 1. Ingredients and baking procedure

	White wheat bread	Whole-grain wheat bread
Flour (g w.w)	980	910
H ₂ O (g)	600	600
Yeast, <i>Sacch., cerevisiae.</i> (g w.w)	50	50
NaCl (g)	6	6
Fermentation (min)	45 + 25	60 + 25
Dough weight (g w.w)	1540	1460
Oven temperature (°C)	200	200
Baking time (min)	40	40
Bread weight (g w.w)	1385	1290

Table 2. Extrusion conditions during processing of crisp-bread products

	White wheat product	Whole-grain wheat product
Mass temperature ^{a)} (°C)	165	159
Feed moisture content (%)	11	13
Feed rate ^{b)} (g w.w./min)	427	457
Screw speed (rpm)	150	150
Pressure ^{a)} (Psi)	$1.9 \cdot 10^3$	$1.1 \cdot 10^3$

Creusot-Loire BC45 twin-screw extruder

Screw configuration; (co-rotating) Feed-CCCCR-Die, C Compression screw element, R Reverse flight screw element

Die geometry; width 20 mm, height 2 mm and length 27 mm

Set temperature of barrel; 90°C

a) mass temperature and pressure was measured with a Dynisco probe in the compression chamber just before the dies.

b) 0.5 % NaCl v/w added

Table 3. Composition of test meals (g/meal)

Breakfast	Carbohydrates Starch	Lactose	Dietary fibre	Protein	Fat	Energy [*] (Kj)
Whole-grain bread	36.3	5.4	8.9	15.7	13.9	1710
White bread	35.9	5.5	2.1	14.6	13.1	1640
Whole-grain extruded	36.0	5.4	8.4	15.7	13.7	1650
White extruded	34.5	5.5	1.6	14.7	13.1	1620

* assuming no energy value of dietary fibre

Table 4. Clinical data

Patient	Sex and age (yrs)	Body mass index (kg/m ²)	Glycosylated haemoglobin HbA1 (%) (n=4)	Fasting blood glucose level (mmol/l) (N=4)	Fasting plasma IRI (nmol/l) (n=4)	Fasting plasma C-peptide (nmol/l) (n=4)
			$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
1	M 51	30 $\pm$ 0	10.8 $\pm$ 0.8	9.2 $\pm$ 1	0.22 $\pm$ 0.04	0.8 $\pm$ 0.12
2	M 74	29 $\pm$ 0	10.1 $\pm$ 0.4	8.4 $\pm$ 0.4	0.05 $\pm$ 0.02	0.59 $\pm$ 0.06
3	M 62	22 $\pm$ 0	7.6 $\pm$ 0.3	7.1 $\pm$ 0.4	0.05 $\pm$ 0.01	0.64 $\pm$ 0.08
4	F 71	42 $\pm$ 0	12.5 $\pm$ 0.6	10.9 $\pm$ 0.6	0.31 $\pm$ 0.04	1.67 $\pm$ 0.08
5	M 63	24 $\pm$ 0	11.4 $\pm$ 1.3	11.0 $\pm$ 2.9	0.08 $\pm$ 0.04	0.85 $\pm$ 0.07
6	M 43	30.8 $\pm$ 0.5	11.7 $\pm$ 1.4	11.6 $\pm$ 0.9	0.06 $\pm$ 0.04	0.58 $\pm$ 0.20
7	M 71	25 $\pm$ 0	8.9 $\pm$ 0.8	6.7 $\pm$ 0.5	0.14 $\pm$ 0.02	0.60 $\pm$ 0.20
8	F 76	27.3 $\pm$ 0.5	9.4 $\pm$ 0.3	7.5 $\pm$ 0.3	0.17 $\pm$ 0.02	1.13 $\pm$ 0.04
9	F 83	30 $\pm$ 0	10.0 $\pm$ 0.4	7.6 $\pm$ 0.4	0.40 $\pm$ 0.24	2.07 $\pm$ 0.07
Mean $\pm$ SEM	63 $\pm$ 4.2	29.0 $\pm$ 1.9	10.3 $\pm$ 0.5	8.9 $\pm$ 0.6	0.16 $\pm$ 0.04	0.99 $\pm$ 0.18

Table 5. Chemical analysis of oven baked and extruded bread products from wheat (% dry basis)

	Starch	Dietary fibre	Protein	Fat
Baked white bread	75.8	4.5	10.0	2.3
Extruded white product	76.4	3.6	10.9	2.5
Baked whole-grain bread	59.7	14.7	11.5	4.0
Extruded whole-grain product	63.5	14.8	12.0	3.9

Table 6. Statistical evaluation of differences at specific time intervals after the test meals (Δ -values)

	WgB - WB	WgE - WgB	WE - WB
Glucose	ns	ns	135' WE > WB (p<0.05)
Insulin		150' WgE > WgB (p<0.01)	ns
C-peptide	ns	150' WgE > WgB (p<0.01)	ns
GIP	ns	30' WgE > WgB (p<0.05)	120' WE > WB (p<0.05)
Glucagon	ns	45' WgE > WgB (p<0.05) 60' WgE > WgB (p0.01)	ns
Triglycerides	ns	ns	ns
Glycerol	ns	ns	ns
Glucose (AUC, 0-180 min)	ns	WgE > WgB (p<0.05)	ns
Insulin (AUC, 0-180 min)	ns	WgE > WgB (p<0.05)	ns
WgB Whole-grain bread			
WB White bread			
WgE Whole-grain extruded			
WE White extruded			
AUC Area under the curve			

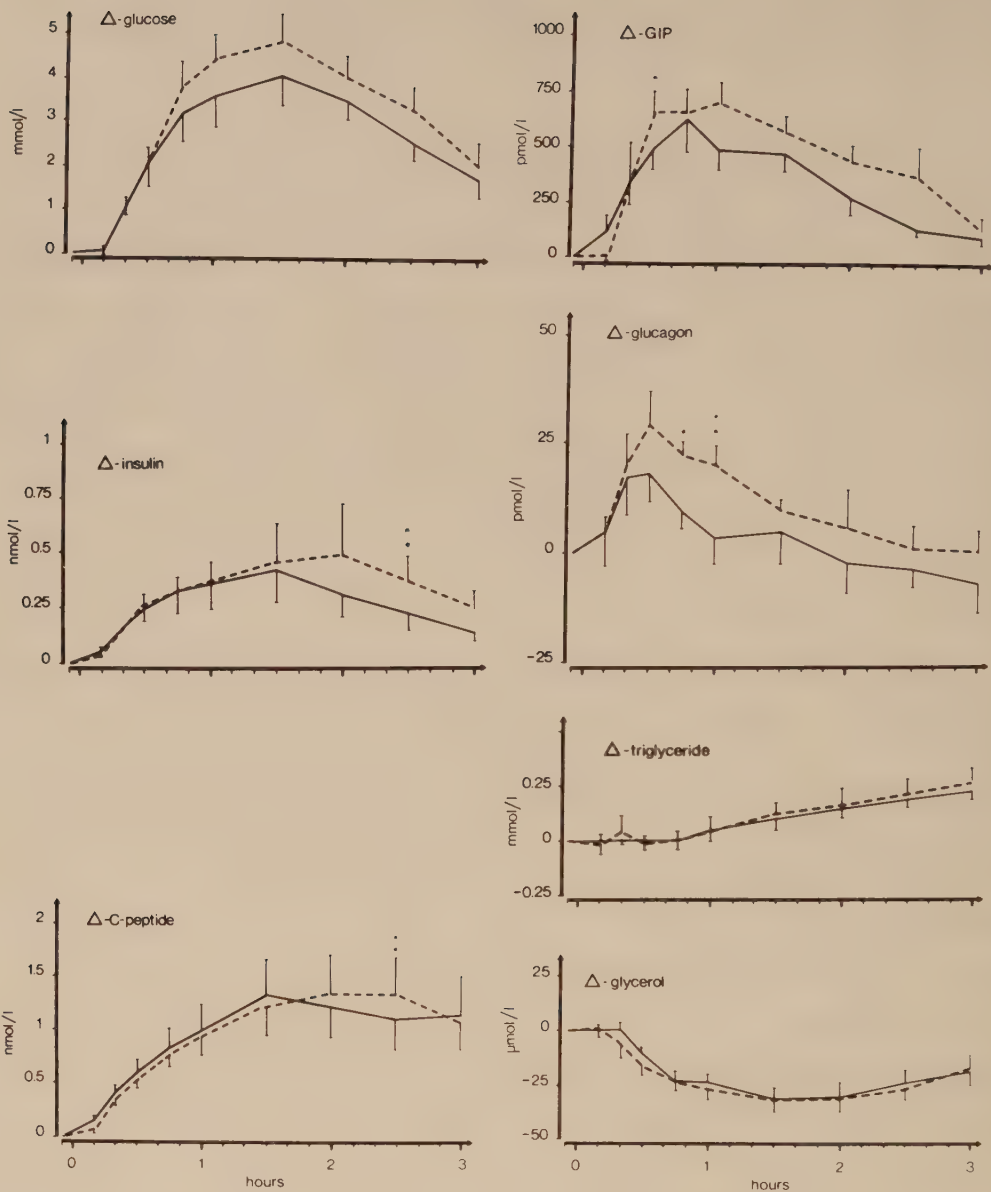


Fig. 1.

Effects of breakfasts with baked whole-grain wheat bread, WgB, (—) and the corresponding extruded product, WgE (----) on the concentrations of blood glucose, plasma insulin, C-peptide, GIP, glucagon, triglycerides and glycerol in nine non-insulin-dependent diabetics. Results are expressed as deviations (Δ) from fasting values. (Mean values $\pm$ SEM,

* $p < 0.05$, ** $p < 0.01$).

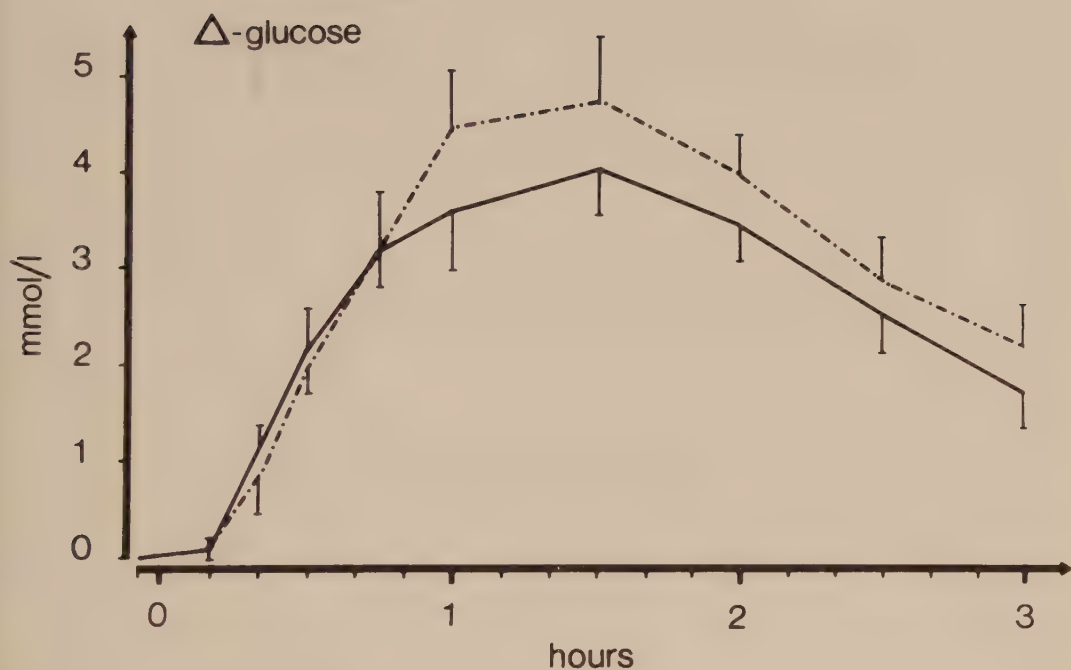


Fig. 2.

Effects of breakfasts with baked whole-grain wheat bread, WgB, (—) and baked white wheat bread, WB, (-·-·-) on the concentrations of blood glucose in nine non-insulin-dependent diabetics. Results are expressed as deviations (Δ) from fasting values. (Mean values $\pm$ SEM).

VI

EXTRUSION COOKING AND DIETARY FIBER :
EFFECTS ON DIETARY FIBER CONTENT AND ON DEGRADATION
IN THE RAT INTESTINAL TRACT

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Cereal Chemistry (in press)

ABSTRACT

Dietary fiber content in raw and extruded wheat products was measured gravimetrically after enzymatic solubilization of protein and starch. There was a slight increase in dietary fiber content after extrusion cooking of whole grain wheat flour and after processing of wheat flour at severe conditions. When the heat-stable α -amylase Termamyl, used in the dietary fiber assay, was excluded, a more pronounced increase in dietary fiber was found. A redistribution of insoluble to soluble dietary fiber was observed in all extruded wheat flour samples. In raw flour 40% was soluble versus 50-75% in extruded wheat flours. In whole grain flour the redistribution was less pronounced. The degradation of dietary fiber monomers in the rat intestine was very similar with raw and extruded whole grain wheat flour. However, in extruded wheat flour, the dietary fiber was more extensively degraded than in the corresponding raw material. There was no increase in fecal glucan excretion when feeding extruded materials, indicating that altered starch was completely absorbed or fermented. Rats fed wheat flour extruded under severe conditions developed diarrhea.

INTRODUCTION

Extrusion-cooking is increasingly used for processing cereal based foods.

Cereals are an important source of dietary fiber, constituting about 40% of the total dietary fiber intake in Sweden (Arnbjörnsson et al 1982). A number of physiological effects have been attributed to dietary fiber, such as increased fecal volume, reduced transit time, improved glucose tolerance and lowered plasma lipids (for review see Vahouny and Kritchevsky, 1982). However, very few data are available on the effects of different food processes on dietary fiber.

Many studies have been performed relating physiological effects to the content and chemical composition of fiber (Jenkins et al 1978, Jenkins 1979). In addition, the physical state of fiber is important. For instance, the bile salt binding capacity decreases with decreasing particle size (Mongeau and Brassard 1982). Further, milling of cereals has been shown to increase the availability of digestible carbohydrates both in-vitro (Greenberg 1976) and in-vivo (O'Dea et al 1980). On the other hand, extrusion-cooked wheat bran was no less beneficial to glucose excretion in alloxandibabetic rats, compared with raw bran (Nygren et al. 1982)¹.

¹ Nygren, C., Hallmans, G., Jonsson, L. and Asp, N-G. 1982. Effects of processed rye and wheat bran on the glucose metabolism. Poster presented at: Intern. Symp. Fiber in human and animal nutrition. Palmerston North, New Zealand, Abstract No. 176.

Thermal processing can make a fraction of the starch less available to enzymes and thus increase the dietary fiber value (Johansson² et al 1983, Varo et al 1983).

Dietary fiber is by definition resistant to the digestive enzymes in the gastrointestinal tract. However, in the large intestine, fermentation occurs through bacterial enzymes (Nyman and Asp 1982). Different kinds of dietary fiber are fermented to various extent. Solubility, chemical structure and particle size, are factors that can be expected to influence the susceptibility to bacterial degradation.

The importance of the physical state of the fiber in relation to bacterial fermentation was demonstrated by Brodribb and Groves (1978). The susceptibility to fermentation in the colon of human subjects was inversely proportional to the size of the bran particles. In addition, Berg et al (1972) found that the breakdown of cellulose by highly cellulolytic bacteria was proportional to its surface area. Heller et al (1980) also demonstrated the importance of particle size. The moisture content in feces was significantly higher in humans given a coarse bran diet compared with a fine bran diet. Further, the bulking effect of bran has been shown to be less pronounced with cooked bran than with raw (Wyman et al 1976).

² Johansson, C-G., Siljeström, M., and Asp, N-G. (1983) Dietary fibre in bread and corresponding flours - Formation of Resistant Starch. *Z. Lebensm. Unters. Forsch.* (submitted)

Thus, processes that involve heating in combination with homogenization, as extrusion-cooking, could be expected to affect dietary fiber, both in terms of physiological properties and in terms of fiber analyses. A variety of products such as pre-gelatinized flours, breakfast cereals, crisp-breads and weaning foods are produced by high-temperature-short-time (HTST) extrusion-cooking. In the extruder, the raw material is subjected to intense mechanical shear through the action of rotating screws. The cooking takes place at high temperature and pressure and at a low water content. The mechanical treatment leads to a complete disorganization of the original structure in the raw material. The new structure obtained has been referred to as a uniform plasticized dough (Kervinen et al 1981). Technical reviews on extrusion-cooking have been published by Harper (1981) and by Linko et al (1981).

The purpose of the present investigation was to study the effect of extrusion-cooking on dietary fiber in wheat products. The dietary fiber content in raw and extruded products was measured gravimetrically after enzymatic solubilization of protein and starch. As all methods for determination of dietary fiber are dependent upon a complete digestion of starch, the efficiency of different enzyme systems for starch degradation was also evaluated. Further, the influence of extrusion-cooking on the susceptibility of dietary fiber to bacterial fermentation was studied through balance experiments in rats.

MATERIALS AND METHODS

Materials

The following materials were used: wheat starch (A-starch, Raisio Factories Ltd, Finland), wheat flour (extraction rate about 80%) and whole grain wheat flour (Vaasa Mills Ltd, Finland). The products were processed in a Creusot-Loire BC 45 twin-screw extruder under conditions described in Table I. The feed rate was 200 g/min except in one case where a higher feed rate was used. Feed moisture content was 15 or 20%. Screw speed was varied in the interval 100-200 rpm. Set temperature of barrel was 150°C in all experiments. Mass temperature was in the interval 161-180°C, and was measured with a thermo-couple inserted just before the die. The extruded materials and the raw whole-grain wheat flour were ground to pass a 0.5 mm sieve. The extruded products had the shape of expanded ribbons and the grinding was performed to enable comparison with the corresponding raw materials. As judged from the degree of browning the most severely processed wheat flour (WF4) must be considered over-cooked.

Methods

Determination of dietary fiber. The dietary fiber content was analyzed gravimetrically after enzymatic solubilization of protein and starch. Two different enzymatic systems for starch degradation was used. One was based on the method by Hellendoorn et al (1975) using only physiological enzymes. After gelatinization for 15 min at 100 C, enzyme digestion was performed with pepsin at pH 1.5 for 1 hr and with pancreatin at pH 6.8 for 1 hr (pepsin, Merck No 7190, and pancreatin, Sigma No P-1750). The dietary fiber was precipitated with 4 vol. of 95% ethanol for 1 hr. Filtration in G-2 crucibles with Celite (0.5 g) as a filtering aid was used to separate the dietary fiber. The other method was described by Asp et al (1983). In addition to incubation with physiological enzymes as above, a thermostable bacterial α -amylase (Termamyl 120L, Novo A/S, Denmark) was added in the gelatinization step. Insoluble fiber was recovered by filtration (G-2 crucibles, Celite as filtering aid). Soluble fiber was precipitated with 4 vol. 95% ethanol and recovered by another filtration.

The dietary fiber residues were corrected for remaining protein (assayed with the Kjeldahl procedure and calculated as $N \times 6.25$) and ash. All analyses were done in triplicates.

Gas-liquid chromatographic characterization of dietary fiber constituents. After acid hydrolysis gas-liquid chromatography (GLC) of the neutral sugars as their alditol acetates was performed as described by Theander and Aman (1979).

The hydrolysis was performed in 12M H_2SO_4 for 2 hrs.

The acid was then diluted to 0.36M and the hydrolysis continued by reflux boiling for 6 hrs. Dietary fiber constituents were expressed as their polymer weights (0.9 x monomer weight).

Starch determination. Approximately 100 mg sample was suspended in 1 ml of distilled water and gelatinized in a boiling water bath for 15 min. One-half ml of 0.2 M sodium-acetate buffer, pH 4.75, and 5 ul of amyloglucosidase (Boehringer Mannheim GMBH, suspension 10 mg/ml) were added. The suspension was incubated at 60°C for 30 min. After centrifugation of the suspension at 4000 rpm, the released glucose was determined with glucose oxidase peroxidase reagent [5.6 g of GLOX-Novum (Kabi-Diagnostica, Stockholm, Sweden) in 100 ml of 0.5 M TRIS buffer, pH 7.0]. The glucose oxidase incubation was performed at 37°C for 1 hr, and the absorbance was measured at 435 nm. Starch content was expressed as glucose x 0.9.

In some determinations 5 ul Termamyl were added in the gelatinization step.

Paper-chromatography. Ten gram flour (dry matter) or 2 g feces (dry matter) were extracted over-night in 80% (v/v) ethanol. The extract was filtered, evaporated and dissolved in 10 ml of distilled water. Paper-chromatography was performed on Whatman No. 3 paper. The following solvent system was used: ethylacetate/acetic acid/water, 3:1:1(v/v/v). Papers were stained with a silver dip reagent (Trevelyan et al 1950). Xylose, glucose, fructose and maltose (50 ug) were used as standards.

Animal experiments

Animals and diets. Male, Sprague-Dawley rats weighing approximately 75 g were divided into groups of five and placed individually in metabolic cages. Food intake was restricted to 10 g dry weight per day. Water was provided ad lib. After a 4 d adaption period, feed residues and feces were collected during a 5 d balance period (Nyman and Asp 1982). Feces were collected dry, removed every day and frozen, -20°C . Feces were then lyophilized, weighed and milled to pass a 0.4 mm screen, and stored at -20°C until analysis.

All diets contained (%): corn-oil 5, sucrose 10, minerals 4.8 and vitamins, including cholin chloride, 1.0 (Table II).

In the case of wheat flour and whole grain wheat flour, the flour was used as protein source, 1.5% N on dry matter. Sucrose was added to adjust dry matter content. In the groups fed wheat starch, the protein source was casein (ANRC reference protein, ICN Nutritional Biochemicals) 1.5% N on dry matter (Table II).

Dietary fiber characterization. Dietary fiber composition in feed and in feces was analyzed by gas-liquid-chromatography as described above. The dietary fiber in feed was isolated by using only physiological enzymes. Analysis of dietary fiber constituents in feces were performed directly on pooled samples from five rats. Glucose values were corrected for the small amounts of free glucose and starch found in feces (approximately 15% of total glucose excretion).

RESULTS

Starch solubilization and dietary fiber content

In Table III different enzyme systems for solubilization of starch have been compared for dietary fiber determination in raw and extruded wheat products (samples WF1 and WGF3). When using only physiological enzymes the dietary fiber content in extruded products was higher. The increment relative raw material was 2.3% and 6.0% in extruded wheat flour and whole grain wheat flour, respectively. After correction for remaining starch with amyloglucosidase, the increase in fiber content due to extrusion was reduced to 1% in the case of wheat flour and to 2.7% with whole grain wheat flour. With Termamyl present in the gelatinization step during starch analysis, corrected dietary fiber values in wheat flour were similar before and after extrusion, about 4%. In extruded whole grain wheat flour the dietary fiber content was still slightly higher, 13.5%, than in the raw material, 12.2%.

Dietary fiber content after pre-incubation with Termamyl

Wheat flour. The content of total, insoluble and soluble dietary fiber analyzed according to Asp et al (1983) is shown in Table IV. In sample WF1, WF2 and WF3 the total dietary fiber content was not significantly different from that of the raw material. In the most severely processed sample (WF4) total dietary fiber increased from 4.0 to 4.9%. This could be accounted for as starch, analyzed by extensive amyloglucosidase digestion. This altered starch was found in the soluble dietary fiber fraction.

Extrusion-cooking caused a redistribution of insoluble to soluble dietary fiber. Thus, in raw wheat flour 40% of total fiber was soluble versus 50-75% in the extruded products. Even in the product processed under mild conditions (WF1) the redistribution was statistically significant.

Gas-liquid chromatographic analysis of fiber constituents confirmed the solubilization observed in the gravimetric assay. Results with the most severely processed product (WF4) and the corresponding raw material are shown in Table V. The different fiber constituents were solubilized to approximately the same extent.

Whole grain wheat flour. The content of total dietary fiber analyzed according to Asp et al (1983) was somewhat higher in all the extruded whole grain wheat flours than in the raw material (Table VI). Only minute amounts of remaining starch could be detected in the fiber residues. The increase of about 1% occurred in the soluble fiber fraction. In two of the samples there was also a slight increase in insoluble fiber. In raw whole grain wheat flour 15% of the total fiber was soluble versus about 20% after processing.

Degradation of dietary fiber in the rat intestinal tract

Wheat starch. Except for glucose, the excretion of typical fiber constituents in feces of rats given a fiberfree diet with wheat starch was very low, < 10 mg/5 days. The fecal excretion

of polyglucose was 51 mg with raw wheat starch, and somewhat higher, 84 mg with extruded wheat starch. However, calculated on ingested starch, there was a complete digestion of both raw and extruded wheat starch, 99.8 and 99.7%, respectively.

Wheat flour. The content of dietary fiber constituents measured with GLC was very similar in raw and extruded wheat flour in the case of arabinose, xylose, mannose and galactose (Table VII). However, the content of glucans in the fiber fraction, i.e. sum of fiber glucose and starch resistant to enzymatic degradation in-vitro, was highly dependent on the enzyme system used for solubilization of starch, as shown in Table III. This was particularly evident with extruded materials. When using only physiological enzymes, the glucan content in extruded wheat flour was 5.3% compared to only 2.9% in the raw material. When corrected for remaining starch available to amyloglucosidase, the glucan content decreased but the level in the extruded product was still higher than in the raw material. With Termamyl present in the starch assay similar glucan values were obtained before and after extrusion.

Dietary fiber in wheat flour was very susceptible to bacterial degradation in the rat intestine (Table VII). When feeding extruded wheat flour the fecal excretion of dietary fiber constituents was even lower than after raw flour, indicating a higher fermentability due to extrusion. The fecal recovery of the main components, arabinose, xylose and glucose averaged 22% in raw wheat flour versus 12% after extrusion.

The balance experiment was performed on wheat flour extruded under mild conditions (WF1). The most severely processed flour (WF4) gave diarrhea, making it impossible to collect feces. To study if this process-induced diarrhea was due to formation of undigestible oligosaccharides, paper-chromatography of ethanol extracts was performed with flour and with feces from rats given raw and extruded wheat flours (WF1 and WF4). In extruded flours, a spot was observed that could not be detected in raw flour. The intensity of the spot was much weaker with the flour processed under mild conditions (WF1). The Rf value was close to that of xylose. Further, an increased content of fructose could be detected in extruded materials. In feces, very similar patterns were observed with all materials. Whole grain wheat flour. The dietary fiber composition measured with GLC was similar in raw and extruded products, except for glucose (Table VIII). Just as with wheat flour, the content of glucans was dependent on the enzyme system used for solubilization of starch. With Termamyl present, the glucan content was similar in raw and extruded materials.

The fecal excretion in mg of dietary fiber constituents, including glucose, was approximately the same before and after processing. Calculated as per cent of intake, the fecal excretion of arabinose was 45% and that of xylose 33%. If intake was corrected for remaining starch with Termamyl, the excretion of glucans was also similar, 46 and 52%, respectively.

DISCUSSION

Depending on the enzyme system used for solubilization of starch different dietary fiber values were obtained in both raw and extruded products. The variability in fiber content was most pronounced after extrusion-cooking. This was directly related to the glucan content in the fiber residues.

Without Termamyl the glucan content in extruded products was considerably higher than in the corresponding raw material. In contrast, with Termamyl present the increase in glucan content due to processing was less pronounced. This could be due to the presence of amylose-lipid complexes in extruded products. Formation of amylose-lipid complexes has been reported during extrusion-cooking. According to Mercier (1980) these complexes are resistant to α -amylase during in-vitro incubation at 37°C. However, Holm et al (1983) recently showed that amylose-lysolecithin complexes were completely digested and absorbed in the rat small intestine. Physiologically, these complexes should therefore be looked upon as starch rather than dietary fiber. Further, it was demonstrated that amylose-lipid complexes were efficiently hydrolyzed by Termamyl. The complexes were also available to pancreatic α -amylase, but high enzyme levels and a long incubation time was needed to obtain complete digestion. The high efficiency of the thermostable α -amylase Termamyl is probably due to dissociation of the amylose-lipid complexes at high temperature (Ghiasi et al 1982), thus increasing the susceptibility for enzymatic attack. When using Termamyl in the analytical procedure for determination

of dietary fiber (Asp et al 1983, Theander and Åman 1979) artifacts due to insufficient digestion of starch in-vitro are therefore minimized. This point is particularly important when analyzing processed products.

The rat balance experiments indicated that the starch digestion was very efficient in-vivo. The glucan content in feces of rats given extruded products was similar to that obtained with the corresponding raw materials. However, the technique used in the balance experiments does not allow us to distinguish between digestion and absorption in the small intestine and bacterial fermentation. It is interesting to notice that the fecal excretion of glucans with wheat flour was only slightly higher than that obtained with raw wheat starch.

In the present study, a solubilization of dietary fiber was observed during extrusion cooking of wheat flour. The solubilization appears to be dependent on process conditions. In the wheat product (WF1) processed at a high feed rate, that is at a shorter residence time, the solubilization was less pronounced. Varo et al (1983) compared different methods for the determination of dietary fiber in processed materials. With some of these methods a redistribution between soluble and insoluble dietary fiber was observed in extruded wheat flour and whole grain wheat flour. However, when results from all methods were combined, the redistribution was not significant. Thus, the solubilization effect was method dependent.

The digestibility of both raw and extrusion cooked wheat starch was almost 100%. Only minute amounts of starch could be detected in rat feces with amyloglucosidase. Thus, extrusion-cooking of pure wheat starch did not affect its digestibility. The excretion of polyglucose on a fiber-free wheat starch diet was 51 mg/5 d. This is in good agreement with the basal excretion obtained with maize starch by Nyman and Asp (1982).

The balance experiments indicated that the dietary fiber in raw wheat flour was fermented to a high degree. However, extrusion-cooking of wheat flour increased the fermentability further. No such effect was obtained with extruded whole grain wheat flour. Our data thus indicate that extrusion-cooking primarily affects the fermentability of the smaller fiber fraction connected with the endosperm.

Food processes that lead to an increase in the surface area of the product could be expected to increase the availability of fiber to fermentation. In extruded wheat flour, the solubilization of fiber might be a factor increasing the availability to microorganisms. In general, soluble fiber is more easily fermented than insoluble fiber (Cummings et al 1979, Nyman and Asp 1982). The significant increase in soluble dietary fiber in extruded wheat flour products could be interpreted as breaking of chemical bonds. Degradation of fiber has been reported with other processes that involve shear at low moisture content. Thus, according to Assarsson et al (1959) glycosidic bonds in cellulose may be broken during dry ball milling.

With whole grain wheat flour the content of dietary fiber constituents in feces was very similar before and after extrusion. This indicates that certain chemical structures are resistant to bacterial degradation in the large intestine in spite of the prominent homogenization during extrusion. These results are in agreement with a recent study (Nyman and Asp 1983)² in which the particle size per se did not affect the fermentation of purified wheat bran in the rat.

Paper chromatography on raw and extruded wheat flour (WF1, WF4) indicated an increase in fructose content due to processing. This is in agreement with earlier results with extrusion-cooked wheat flour (Chiang and Johnson 1977). The diarrhea observed in rats given severely processed wheat flour, cannot be explained at present. Maillard-reaction products formed during heat treatment by a reaction between proteins and reducing sugars have not been reported to cause diarrhea.

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² Nyman, M. and Asp, N-G. Unpublished results. Department of Food Chemistry, University of Lund, Lund, Sweden

LITERATURE CITED

- ARNBJÖRNSSON, E., ASP, N-G., and WESTIN, S.I. 1982.
Decreasing incidence of acute appendicitis, with special
reference to the consumption of dietary fiber.
Acta Chir Scand 148:461.
- ASP, N-G. JOHANSSON, C-G., HALLMER, H., and SILJESTRÖM, M. 1983.
Rapid enzymatic assay of insoluble and soluble dietary fiber.
J. Agric. Food Chem. 31:476.
- ASSARSSON, A., LINDBERG, B., and THEANDER, O. 1959. Studies on
mechanically degraded cellulose. *Acta Chem. Scand.* 13:1231.
- BERG, B., v. HOFSTEN, B., and PETTERSSON, G. 1972.
Electronmicroscopic observations on the degradation of cellulose
fibres by *cellvibrio fulvus* and *sporocytophaga myxococcoides*.
J. app. Bact. 35:215.
- BRODRIBB, A.J.M., and GROVES, C. 1978. Effect of bran particle
size on stool weight. *Gut* 19:60.
- CHIANG, B-Y., and JOHNSON, J.A. 1977. Gelatinization of starch
in extruded products. *Cereal Chem.* 54:436.
- CUMMINGS, J.H., SOUTHGATE, D.A.T., BRANCH, W.J., WIGGINS, H.S.,
HOUSTON, H., JENKINS, D.J.A., JIVRAJ, T., and HILL, M.J. 1979.
The digestion of pectin in the human gut and its effect on
calcium absorption and large bowel function. *Br. J. Nutr.* 41:477.
- GHIASI, K., HOSENEY, R.C., and VARRIANO-MARSTON, E. 1982.
Gelatinization of wheat-starch. I. Excess-water systems.
Cereal Chem. 59:81.
- GREENBERG, C.J. 1976. Studies on the fibre in human diets and
its effect on the digestion and absorption of other nutrients.
Dissertation, University of Cambridge, England.
- HARPER, J.M. 1981. Extrusion of foods. Vol I and II.
CRC Press Inc: Boca Raton, Florida, USA.
- HELLENDORRN, E.W., NOORDHOFF, M.G., and SLAGMAN, J. 1975.
Enzymatic determination of the indigestible residue (dietary
fibre) content of human food. *J. Sci. Fd. Agric.* 26:1461.

- HELLER, S.N., HACKLER, L.R., RIVERS, J.M., VAN SOEST, P.J., ROE, D.A., LEWIS, B.A., and ROBERTSON, J. 1980. Dietary fiber: the effect of particle size of wheat bran on colonic function in young adult men. *Am. J. Clin. Nutr.* 33:1734.
- HOLM, J., BJÖRCK, I., OSTROWSKA, S., ELIASSON, A-C., ASP N-G., LARSSON, K., and LUNDQUIST, I. 1983. Digestibility of amylose-lipid complexes in-vitro and in-vitro. *Stärke*, 35:294.
- JENKINS, D.J.A., WOLEVER, T.M.S., LEEDS, A.R., GASSULL, M.A., HAISMAN, P., DILAWARI, J., GOFF, D.V., METZ, G.L., and ALBERTI, K.G.M. 1978. Dietary fibres, fibre analogues, and glucose tolerance: Importance of viscosity. *Br. Med. J.* 1:1392.
- JENKINS, D.J.A. 1979. Dietary fibre, diabetes and hyperlipidaemia. Progress and Prospects. *The Lancet* 15:1288.
- KERVINEN, R., LINKO, P., and OLKKU, J. 1981. Extrusion cooking induced changes in functional properties of wheat flour. Page 219 in: *Nordisk Cerealistförbunds 21:a kongress, Espoo, 9-11 juni.*
- LINKO, P., COLONNA, P., and MERCIER, C. 1981. High-temperature, short-time extrusion cooking. Page 145 in: Pomeranz, Y., ed. *Advances in Cereal Science and Technology*, vol IV. American Association of Cereal Chemists Inc, St. Paul: Minnesota, USA.
- MERCIER, C. 1980. Structure and digestibility alterations of cereal starches by twin-screw extrusion cooking. Page 795 in: Linko, P., Malkki, Y., Olkku, J., and Larinkari, J., eds. *Food Process Engineering*. Applied Science Publishers Ltd: London.
- MONGEAU, R., and BRASSARD, R. 1982. Insoluble dietary fiber from breakfast cereals and brans: Bile salt binding and water-holding capacity in relation to particle size. *Cereal Chem.* 59:413.
- NYMAN, M., and ASP, N-G. 1982. Fermentation of dietary fibre components in the rat intestinal tract. *Br. J. Nutr.* 47:357.
- O'DEA, K., NESTEL, P.J., and ANTONOFF, L. 1980. Physical factors influencing postprandial glucose and insulin responses to starch. *Am. J. Clin. Nutr.* 33:760.
- THEANDER, O., and ÅMAN, P. 1979. Studies on dietary fibres. 1. Analysis and chemical characterization of water soluble and water insoluble dietary fibres. *Swedish J. agric. Res.* 9:97.

- TREVELYAN, W.E., PROCTER, D.P., and HARRISON, J.S. 1950.
Detections of sugars on paper chromatograms, *Nature* 166:444.
- VAHOONY, G.V., and KRITCHEVSKY, D. eds. 1982. *Dietary Fiber in Health and Disease*, Plenum Press: New York and London.
- VARO, P., LAINE, R., and KOIVISTOINEN, P. 1983. Effect of heat treatment on dietary fiber: Interlaboratory study. *J. Assoc. Off. Anal. Chem.* 66:933.
- WYMAN, J.B., HEATON, K.W., MANNING, A.P., and WICKS, A.C.B. 1976. The effect on intestinal transit and the feces of raw and cooked bran in different doses. *Am. J. Clin. Nutr.* 29:1474.

Table I

Process conditions during extrusion-cooking of starch, wheat flour and whole grain wheat flour

	Wheat Starch	Wheat Flour				Whole Grain Wheat Flour		
		WF1	WF2	WF3	WF4	WGF1	WGF2	WGF3
Mass feed rate including moisture (g/min)	200	350	200	200	200	200	200	200
Feed moisture content (%)	15	15	15	15	15	20	20	20
Screw speed (rpm)	200	150	100	150	200	100	150	200
Mass temperature (°C)	180	161	161	171	171	164	166	166

Creusot-Loire BC 45 twin-screw extruder

Set temperature of barrel 150 °C

Die : Die diameter 5mm

Capillary length 27mm

Clearance die/endplate 2mm

Screw : Screw combination Die- RCCCC- Feed

R Reverse flight screw element

C Compression screw element

Co-rotating intermeshing screws

Table 11. Composition of the diets (% dry matter)

Component	Wheat starch ^a		Wheat flour ^b		Whole grain wheat flour ^c	
	Raw	Extruded ^a	Raw	Extruded ^b	Raw	Extruded ^c
Wheat product	69.3	69.3	74.2	76.3	81.6	81.2
Casein	9.9	9.9	-	-	-	-
Sucrose	10.0	10.0	15.0	12.9	7.6	8.0
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0
Minerals ^d	4.8	4.8	4.8	4.8	4.8	4.8
Vitamins ^e	0.8	0.8	0.8	0.8	0.8	0.8
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2

^aProcess conditions described in Table I.

^bFlour WF4 with process conditions described in Table I.

^cFlour WGF3 with process conditions described in Table I.

^dContaining (g): $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ 8.6, $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$ 32.0, KH_2PO_4 7780, $\text{NaH}_2\text{PO}_4 \cdot 2 \text{H}_2\text{O}$ 4024, CaCO_3 7600, KI 1.6, $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ 2000, $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ 18, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 80, CoCl_2 0.46, NaCl 2382.

^eContaining (g): Menadione 25.0, thiaminhydrochloride 10.0, riboflavin 10.0, pyridoxin hydrochloride 5.0, calcium pantothenate 25.0, nicotinic acid 25.0, folic acid 1.0, inositol 50.0, p-aminobenzoic acid 5.0, biotin 0.2, vitamin B12 cyanocobalamin 0.015, vitamin A 0.86, vitamin D 0.025, vitamin E 100, Wheat starch 3765.

TABLE III

Enzymatic gravimetric assay of dietary fiber in raw and extruded flours:

Comparison between different enzyme systems.

Enzyme system	Dietary fiber (% dry matter)			
	Wheat flour		Whole grain wheat flour	
	Raw Mean $\pm$ SD	Extruded (WF1) Mean $\pm$ SD	Raw Mean $\pm$ SD	Extruded (WGF3) Mean $\pm$ SD
Physiological enzymes only (pepsin, pancreatin)	6.8 $\pm$ 0.4	9.1 $\pm$ 0.6 ^{***}	14.5 $\pm$ 0.3	20.5 $\pm$ 0.8 ^{***}
corrected for remaining starch (amylglucosidase)	4.2 $\pm$ 0.4	5.2 $\pm$ 0.6 [*]	12.4 $\pm$ 0.3	15.1 $\pm$ 0.8 ^{**}
corrected for remaining starch (Termamyl, amyl- glucosidase)	4.0 $\pm$ 0.4	3.9 $\pm$ 0.6	12.2 $\pm$ 0.3	13.5 $\pm$ 0.8 [*]

Significantly different from raw material (Student's t-test) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table IV

Dietary fiber content in raw and extruded wheat flours

Material	Dietary fiber ^a (% dry matter)					
	Total		Insoluble		Soluble	
	Mean $\pm$ SD	(Mean $\pm$ SD) ^b	Mean $\pm$ SD	(Mean $\pm$ SD) ^b	Mean $\pm$ SD	(Mean $\pm$ SD) ^b
Raw wheat flour	4.0 $\pm$ 0.2	4.0 $\pm$ 0.2	2.3 $\pm$ 0.1	2.3 $\pm$ 0.1	1.7 $\pm$ 0.1	1.7 $\pm$ 0.1
Extruded products ^c	WF1	3.8 $\pm$ 0.1	3.7 $\pm$ 0.1	1.7 $\pm$ 0.1***	1.7 $\pm$ 0.1***	2.0 $\pm$ 0.1**
	WF2	4.2 $\pm$ 0.5	3.9 $\pm$ 0.5	1.2 $\pm$ 0.2***	1.2 $\pm$ 0.2***	2.7 $\pm$ 0.4***
	WF3	4.3 $\pm$ 0.1	3.6 $\pm$ 0.1*	0.9 $\pm$ 0.1***	0.9 $\pm$ 0.1***	2.7 $\pm$ 0.1***
	WF4	4.9 $\pm$ 0.2***	3.9 $\pm$ 0.2	1.1 $\pm$ 0.3***	1.1 $\pm$ 0.3***	2.8 $\pm$ 0.1***

a Dietary fiber content was measured according to Asp et al (1983).

b Corrected for remaining starch in the fiber residue (amyloglucosidase).

c Process conditions described in Table I.

Significantly different from raw material (Student's t-test) * p<0.05, ** p<0.01 and ***p<0.001.

TABLE V

Composition of dietary fiber in raw and extruded wheat flours

Relative composition(%)	Dietary fiber composition			
	Raw		Extruded (WF4) ^b	
	Insoluble	Soluble	Insoluble	Soluble
Arabinose	20	13	10	26
Xylose	26	16	9	34
Mannose	3	-	3	-
Galactose	-	3	-	6
Glucose	16	3	6	6
Total	65	35	28	72
Sum of polysaccharides (g/100g dry matter)	2.0	1.1	0.9	2.3

^aDietary fiber content was measured according to Asp et al (1983).^bProcess conditions described in Table I.^cCorrected for remaining starch in the fiber residue (amylglucosidase).

TABLE VI

Dietary fiber content in raw and extruded whole grain wheat flours

Material	Dietary fiber ^a (% dry matter)				
	Total		Insoluble		Soluble
	Mean [±] SD	(Mean [±] SD) ^b	Mean [±] SD	(Mean [±] SD) ^b	Mean [±] SD (Mean [±] SD) ^b
Raw whole grain					
wheat flour	13.1 [±] 0.5	13.0 [±] 0.5	11.1 [±] 0.1	11.1 [±] 0.1	2.0 [±] 0.4 1.9 [±] 0.4
Extruded products ^c					
WGF1	14.4 [±] 0.2 [*]	14.1 [±] 0.2 [*]	11.6 [±] 0.2 ^{**}	11.6 [±] 0.2 ^{**}	2.8 [±] 0.2 [*] 2.5 [±] 0.2
WGF2	14.3 [±] 0.2 [*]	14.1 [±] 0.2 [*]	11.4 [±] 0.1 [*]	11.4 [±] 0.1 [*]	2.9 [±] 0.2 [*] 2.7 [±] 0.2 [*]
WGF3	14.2 [±] 0.1 [*]	13.9 [±] 0.1 [*]	10.9 [±] 0.2	10.9 [±] 0.2	3.3 [±] 0.1 ^{**} 3.0 [±] 0.1 [*]

^aDietary fiber content measured according to Asp et al (1983).^bCorrected for remaining starch in the fiber residue (amylglucosidase).^cProcess conditions described in Table I.

Significantly different from raw material (Student's t-test) * p<0.05 and **p<0.01.

TABLE VII

Composition and fecal recovery of dietary fiber in raw and extruded wheat flour (pooled samples from five rats)

	Composition of dietary fiber ^a (% dry matter)		Intake (mg/5d)		Fecal excretion (% of intake)			
	Raw	Extruded ^b	Raw	Extruded ^b	(mg/5d)		Raw	
					Raw	Extruded ^b	Raw	Extruded ^b
Arabinose	0.9	0.8	310	250	57	29	18	12
Xylose	1.1	1.1	390	320	85	34	22	11
Mannose	0.1	0.2	48	45	15	8	31	18
Galactose	0.2	0.2	75	66	34	17	45	26
Glucose	2.9	5.3	990	1590	78	52	7	3
	1.1 ^c	2.4 ^c	360 ^c	710 ^c	78	52	22	7
	0.9 ^d	1.2 ^d	310 ^d	360 ^d	78	52	25	14
Total	3.2 ^d	3.5 ^d	1160 ^d	1130 ^d	270	140	23	12

^aDietary fiber fractions were isolated by using physiological enzymes.^bFlour WF1 with process conditions described in Table I.^cCorrected for remaining starch in the fiber residue (amyloglucosidase).^dCorrected for remaining starch (Termamy1, amyloglucosidase).

TABLE VIII

Composition and fecal recovery of dietary fiber in raw and extruded whole grain wheat flour (pooled samples from five rats)

	Composition of dietary fiber (% of dry matter)		Intake (mg/5d)		Fecal excretion			
	Raw	Extruded ^b	Raw	Extruded ^b	(% of intake)			
					Raw	Extruded ^b	Raw	Extruded ^b
Arabinose	2.9	2.7	1170	1060	530	490	45	46
Xylose	4.5	4.2	1810	1660	600	540	33	33
Mannose	0.2	0.2	61	80	25	24	41	30
Galactose	0.3	0.4	130	140	135	67	104	48
Glucose	5.6	10.0	2280	4000	630	650	28	16
	3.6 ^c	6.4 ^c	1460 ^c	2540 ^c	630	650	43	26
	3.4 ^d	3.1 ^d	1380 ^d	1240 ^d	630	650	46	52
Total	11.3 ^d	10.6 ^d	4550 ^d	4180 ^d	1920	1770	42	42

^aDietary fiber fraction were isolated by using physiological enzymes

^bFlour WGF3 with process conditions described in Table I

^cCorrected for remaining starch in the fiber residue (amyloglucosidase)

^dCorrected for remaining starch in the fiber residue (Termamyl, amyloglucosidase)

